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NUCLEAR DEOXYRIBONUCLEIC ACID CONTENT MEASURED BY STATIC CYTOMETRY: IMPORTANT PROGNOSTIC ASSOCIATION FOR PATIENTS WITH CLINICALLY LOCALIZED PROSTATE CARCINOMA TREATED BY EXTERNAL BEAM RADIOTHERAPY

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ABSTRACT

To determine if relative nuclear deoxyribonucleic acid (DNA) content is an important prognostic parameter for patients with clinically localized prostate carcinoma treated by external beam radiotherapy, we performed static DNA cytometry on archival paraffin embedded prostate needle biopsy specimens obtained before treatment. DNA content was measured with the Zeiss IBAS 2000 Image Analyzer and the Feulgen staining method. Tumor samples from 65 patients with clinically localized carcinoma of the prostate treated with at least 6,000 cGy. from 1974 to 1980 were studied. Patients and tumors were divided into 2 groups: group 1—31 patients with relative DNA content less than 1.5 times normal and group 2—34 patients with relative DNA content greater than 1.5 times normal. The prostate cancer nonprogression rate at 10 years was 64% for group 1 and 11% for group 2. Prostate cancer cause specific survival at 10 years was 73% for group 1 and 20% for group 2. These differences are highly significant ($p < 0.0001$). By contrast, stratification and analysis according to tumor clinical stage, Mayo histological nuclear grade or Gleason score proved not to be as significant. Cox multivariate analysis also identified DNA content as the most important independent variable for cancer specific survival and progression. Nuclear DNA content measured by static cytometry appears useful in identifying those patients with clinically localized prostate carcinoma who may have a favorable probability of long-term disease control by external beam radiotherapy.

KEY WORDS: DNA, radiotherapy, prognosis, cytometry

Nuclear deoxyribonucleic acid (DNA) ploidy measured by flow cytometry has been used to analyze groups of patients with clinically localized carcinoma of the prostate treated by radical prostatectomy and pelvic lymphadenectomy. These studies have shown that patients who have pathological stages B, C and D1 disease with tumor nuclei that contain a normal DNA content (DNA diploid tumors) have a much more favorable prognosis than those who have an increased amount of DNA in the tumor cell nucleus, either DNA tetraploid or DNA aneuploid ploidy patterns.[1-5] Members of our Mayo Clinic research group believe that DNA ploidy, which is now an easily measured objective tumor variable, will become a standard tool for the classification and management of patients with prostate carcinoma treated by radical prostatectomy along with stage and histological grade.

The prognostic association of DNA ploidy for patients with clinically localized carcinoma of the prostate treated by external beam radiotherapy has not been extensively analyzed. In part, this reflects the relative amount of tumor material available for laboratory analysis. For patients treated by radical prostatectomy and pelvic lymphadenectomy the entire primary tumor sample as well as any lymph node metastases are available for archival studies. In contrast, for those patients treated with external beam radiotherapy, usually only small volumes of biopsy material, either needle biopsy cores or transurethral resection chips, are available for study.

For the current investigation we used static DNA cytometry rather than flow cytometry and needle biopsy cores as the tumor substrate. Static cytometry seemed appropriate because relative DNA content could be determined from samples that contained as few as 100 tumor nuclei in the specimen. Moreover, since the nuclei to be analyzed were operator selected, even biopsies that contained a small percentage of tumor nuclei could be studied by this method. Such specimens are not generally suitable for flow cytometric ploidy analysis in which the small number of tumor nuclei could be diluted or camouflaged by the large number of normal stromal cells.

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MATERIALS AND METHODS

We selected 91 patients with clinically localized prostate cancer diagnosed by needle biopsy and treated with at least 6,000 cGy. of external beam irradiation to the prostate area between 1974 and 1980. This study design ensured a minimum followup of 10 years. The radiation therapy technique has been described in detail previously.[6]

The hematoxylin and eosin stained biopsy slides were reviewed by the study pathologist (G. M. F.). Six μm. thick sections of the paraffin embedded tumor needle biopsy core and a normal lymph node for control were mounted side by side on the same slide and stained by the Feulgen method. We excluded 22 cases because of poor staining result or inadequate tumor cell representation in the remaining tissue blocks.

Nuclear DNA content measurements were performed with the Zeiss IBAS 2000 Image Analysis System. The image of the Feulgen stained nucleus of tumor cells to be quantitatively analyzed as displayed on a video monitor is identified by a cursor by an operator. The optical densities of the individual nucleus samples as well as of control lymphocytes are computer stored and then displayed as small histograms. At least 100 tumor nuclei and 20 lymphocytes were measured for each sample. The control normal lymphocytes usually gave a sharp DNA content (optical density) peak that was arbitrarily assigned a relative DNA content of 1.0. The mean DNA content

of the tumor nuclei was divided by the mean tumor DNA content of the normal lymphocytes to obtain the relative tumor DNA content.

Patients were followed on a 3 to 4-month basis for 2 years, then every 6 months for 2 years and annually thereafter. Routine tests included physical examinations, serum acid phosphatase, prostate specific antigen (PSA) when it was available after 1987, chest x-rays, skeletal survey and bone scan, the latter being obtained at 1 year. Evidence of progression was based on biopsy positive local recurrence, positive x-rays or bone scans. Elevated acid phosphatase alone was not considered to be adequate evidence of progression. The histological material and the clinical records of the patient were reviewed without knowledge of the DNA content results. Four cases were excluded due to inadequate followup information, leaving a total of 65 cases with complete clinical followup information as well as adequately analyzable tumor samples, which form the basis of this report.

RESULTS

All 65 patients available for study had complete clinical followup and it was technically possible to perform adequate static DNA measurement of the original prostate needle biopsy cores. Mean patient age was 65.4 years (range 46 to 79 years) and mean followup was 12.4 ± 1.9 years (range 8.9 to 16.1 years). Table 1 lists Mayo nuclear grade, Gleason score, clinical stage and overall clinical status at the time of this report. Of 65 patients 16 (25%) are alive without evidence of disease, 4 (6%) are alive with recurrences, while 32 (49%) are dead from prostate cancer and 13 (20%) are dead from other causes.

Of the 16 disease-free survivors we have recent PSA (Hybritech) information on 9. The PSA values ranged from less than 0.1 to 8.2 (median 1.8). Hormonal treatment was given to 42 patients (65%); while most (71%) were given therapy after the diagnosis of recurrence, 12 patients (29%) received early hormonal treatment, that is before or within 3 months of radiotherapy. Hormonal therapy had been given to 7 of the 16 survivors (47%) compared to 71% of the entire group. The survivors who had received hormonal treatment had a median PSA of 1.8 (range less than 0.1 to 8.2) and those without hormonal treatment had a median PSA of 0.7 (range 0.5 to 2.1). Of the 23 patients who did not receive hormonal treatment 9 (29%) survived without evidence of disease, while 7 of the 42 patients (17%) who received hormonal treatment survived without evidence of recurrence. The difference was not statistically significant ($p = 0.12$), which may reflect the policy of starting hormonal treatment after the diagnosis of recurrence.

The relative mean DNA content of the tumor nuclei measured ranged from 0.97 to 3.13 when compared to normal lymph node lymphocyte nuclei stained on the same slide. As a comparison, the relative DNA content of normal prostate epithelial cells found in hyperplastic glands was 1.19 ± 0.8. Therefore, a normal range of relative DNA content measured by the static cytometric technique used was set at less than 1.5. This range included all DNA measurements that would be less than 3 standard deviations away from that found for normal prostate epithelial cells. Somewhat arbitrarily the tumor population was then divided into 2 groups according to this criterion: group 1—low, relatively normal DNA content, less than 1.5, seen in 31 patients and group 2—abnormally high DNA content 1.5 or greater, found in 34 patients (fig. 1). Both groups were relatively evenly distributed with regard to clinical stage, Mayo histological grade and percentage of adjuvant hormonal treatment (table 2).

Five patients (42%) with Mayo grades 1 and 2 tumors showed normal DNA content, whereas 27 patients (49%) with (high) Mayo grades 3 and 4 tumors showed normal DNA content. The difference between these groups was not statistically significant. Many patients with high grade tumors had tumor nuclei that contained a relatively normal DNA content (fig. 2). Of the (high) Gleason score 8–10 group 10 patients (71%) had an abnormal DNA content, whereas 25 of the Gleason score 5–7 group (52%) had an abnormally elevated DNA content. The difference between these groups was statistically significant

TABLE 1. *Patient and tumor characteristics*

	No.	(%)
Mayo nuclear grade:		
1	1	(2)
2	11	(17)
3	45	(69)
4	8	(12)
Clinical stage:		
A	1	(2)
B	32	(49)
C	32	(49)
Gleason score:		
2–4	3	(5)
5–7	48	(73)
8–10	14	(22)
Current status:		
Alive, no evident disease	16	(25)
Alive with prostate Ca	4	(6)
Dead from prostate Ca	32	(49)
Dead from other causes	13	(20)

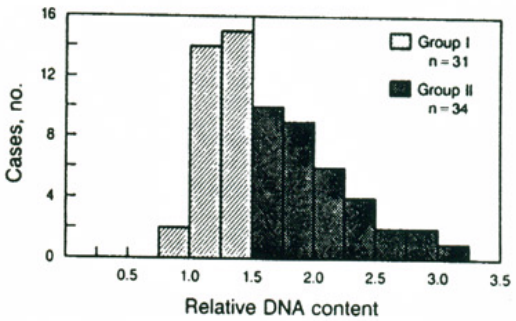

FIG. 1. Mean relative DNA content distribution for prostate cancer biopsy samples studied. Lymph node lymphocytes stained on same slide were given relative values of 1. Prostate epithelial cells found in hyperplastic glands were found to have relative DNA content of 1.19. Group 1 had mean relative DNA content of less than 1.5 and group 2 had mean relative DNA content of 1.5 or greater.

TABLE 2. *Comparison of groups 1 and 2*

	DNA Content Less Than 1.5	DNA Content 1.5 or Greater
Av. age (yrs.)	66.2	64.5
No. clinical stages A and B (%)	18 (58)	15 (44)
No. clinical stage C (%)	13 (42)	19 (56)
No. Mayo grades 1 and 2 (%)	5 (16)	7 (20)
No. Mayo grades 3 and 4 (%)	26 (84)	27 (80)
No. early adjuvant hormonal treatment (%)	11 (35)	10 (29)

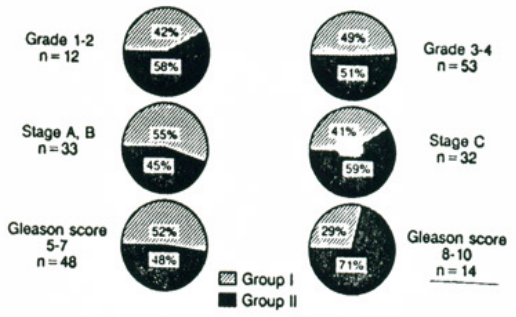

FIG. 2. Distribution of groups 1 and 2 relative DNA content tumors according to high and low Mayo nuclear grades, clinical stages and Gleason scores.

($p < 0.005$, fig. 2). For the low clinical stages A and B tumors 15 of 33 patients (45%) had an abnormally elevated DNA content, whereas 19 of 32 patients (59%) with high stage C tumors had an elevated DNA content. This difference was not statistically significant (fig. 2).

A dramatic difference in cancer progression rate was seen when cases were stratified according to whether the tumor nuclear DNA content was close to normal or elevated. For group 1 patients with a relative DNA content of less than 1.5, 64% had shown no evidence of disease progression when evaluated at 10 years. By contrast, group 2 patients with an elevated relative DNA content showed a much higher progression rate with only 32% disease-free at 5 years and 11% disease-free at 10 years. The difference in the nonprogression curves was highly statistically significant (log rank test, $p < 0.00001$). As might be expected, major differences in disease specific survival were also observed for these groups. Of the patients with relatively normal tumor cell DNA content 75% had not died of prostate cancer 10 years after diagnosis and treatment, which was true for only 27% of those with elevated DNA content. Thus, in summary, separation of tumors according to the tumor cell relative DNA content provided a significant stratification when patients were analyzed for subsequent disease progression and death from prostate cancer.

Kaplan-Meier curves, which show the relationship between disease progression and cause specific survival analyzed according to the initial clinical stage, Mayo nuclear grading and Gleason pattern are presented in figure 3. As might be expected, those patients with clinical stage C tumors had a poorer prognosis than those with clinical stages A and B tumors. Those patients with high Gleason score tumors had a poorer prognosis than those with low Gleason score tumors. Those patients with higher Mayo nuclear grade tumors had a poorer prognosis than those with low Mayo grade scores. As is readily apparent in comparing these figures with the corresponding Kaplan-Meier curves for progression and cause specific survival according to DNA content (fig. 4), none of these traditional measures of tumor behavior comes close to having the same predictive association as does DNA content (or DNA ploidy). This finding was confirmed on Cox multivariate analysis for cancer specific survival and cancer nonprogression. DNA content grouping stood out as the most significant independent variable for both.

DISCUSSION

The data presented in this report indicate that relative DNA content of tumor cell nuclei can be reliably measured from prostate needle biopsy specimens removed by Vim-Silverman biopsy needles 10 to 15 years previously. Although the resultant histograms, which are obtained from approximately 100 nuclei, are not nearly as smooth or convincing as flow cytometric DNA

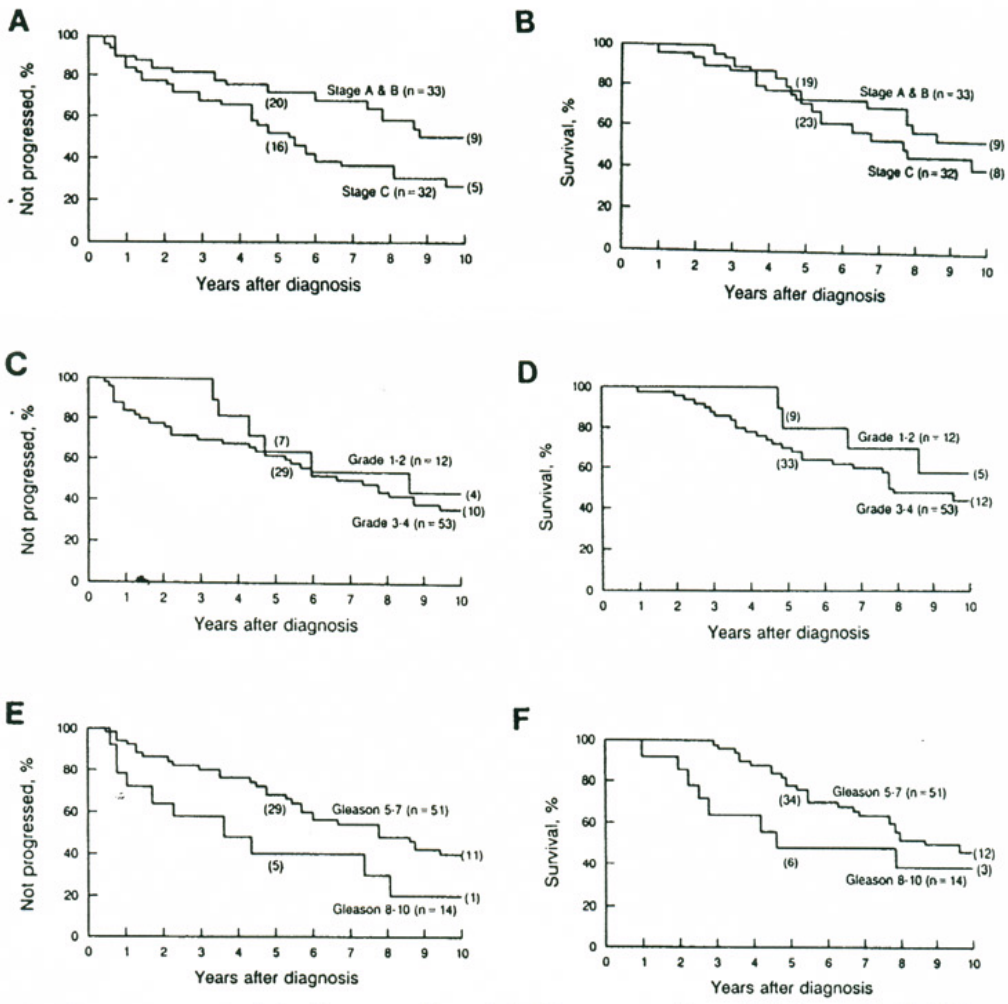

FIG. 3. Kaplan-Meier nonprogression (*A*) and cause specific survival (*B*) curves according to clinical stages. Kaplan-Meier nonprogression (*C*) and cause specific survival (*D*) curves according to Mayo nuclear grading system. Kaplan-Meier nonprogression (*E*) and cause specific survival (*F*) curves according to Gleason score.

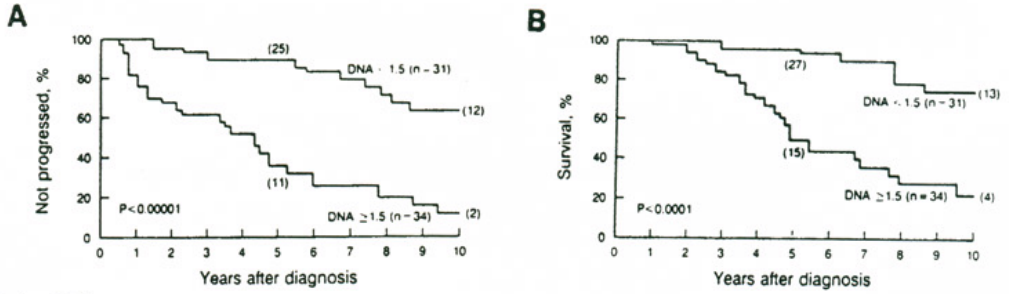

FIG. 4. Kaplan-Meier nonprogression (*A*) and cause specific survival (*B*) curves according to tumor cell relative DNA content

histograms of 20,000 to 50,000 nuclei, it is still possible to make a crude classification as to whether the tumor cell nuclei have a relatively normal DNA content compared to control lymphocytes (less than 1.5) or a higher DNA content (1.5 or greater). With our technique we do not believe it is possible to separate tetraploid and nontetrapolid-aneuploid cells such as can be reliably done by flow cytometry with a large number of sample observations.

Our research group has previously observed that patients with pathological stages B, C and D1 prostate cancer treated by radical prostatectomy have a much more favorable prognosis if the tumors are DNA diploid compared to if they have an abnormal DNA content, either DNA tetraploid or DNA aneuploid. The present study extends these observations to patients with apparently localized carcinoma of the prostate who were treated with external beam radiotherapy (6,000 cGy. or more) many years ago. For these patients, studied in this case by static DNA cytometry, marked difference was seen in tumor progression rates and cause specific survival when tumors were separated somewhat arbitrarily into higher and lower DNA content groups. The results suggest that patients with relatively normal DNA content tumors have a much higher probability of responding favorably to external beam radiotherapy than those with higher DNA content tumors. Of course, these results are based on a relatively small (65) group of patients. Since this type of DNA analysis can be performed on archival tissue, it should be possible for others who institutionally have a large group of patients treated by external beam or interstitial radiation therapy to attempt to confirm these results.

Of considerable interest is the fact that abnormal high DNA content by static cytometry could be found in low and high grade tumors and in low and high stage tumors. None of these more traditional ways of assessing tumor aggressiveness, that is clinical stage, Mayo nuclear score or Gleason score, was nearly as potent as static DNA content in predicting patient response to external beam radiotherapy. Members of our research group are convinced that static DNA cytometry technically can be reliably performed even on small Biopty* gun prostate biopsies that contain few tumor nuclei. Static cytometry allows individual tumor cell nuclei to be detected by the observer and nontumor nuclei, which could camouflage results, to be rejected from study. This observer interaction with the tissue image is a great advantage over flow cytometric analysis for tiny biopsy specimens or for those that contain a small percentage of tumor nuclei. Conversely, it is much more time-consuming and labor intensive than flow cytometric ploidy analysis using the Hedley technique, which can be easily automated for large numbers of samples.

Members of our research group currently believe that flow cytometric ploidy analysis gives the single most objective and important parameter associated with prognosis for patients with localized prostate carcinoma treated by radical prostatectomy. The current data suggest that static cytometry may yield uniquely important prognostic associations for patients treated by external beam radiotherapy. Although both methodologies must be somewhat beset by sampling errors within the large volume of prostate tumor cells available, the consistency of the results found, in terms of subsequent patient behavior, suggest that nuclear DNA content for prostate tumor cells must be a relatively stable genotypic-phenotypic feature for these cancers, at least for the clinical stages being investigated. Additional supportive published evidence for the prognostic importance of DNA ploidy in early stage tumors treated by observation[7] and in stage D1 tumors treated by ^{125}iodine seed implantation has also been published.[8] Finally, the favorable prognosis found for patients with low DNA content tumors may simply reflect the relatively good natural history for such DNA diploid prostate cancers rather than the radiotherapy treatment. The retrospective experimental design of this study did not include a control group of patients who did not receive radiotherapy but it appears certain that those patients with high DNA content tumors were not well treated by external beam radiotherapy.

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