Near Tetraploid Prostate Carcinoma

Methodologic and Prognostic Aspects

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Supported by grants from the Swedish Cancer Society, the Cancer Society in Stockholm, and funds from the Karolinska Institute.

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Received April 1, 1996; revision received June 26, 1996, accepted June 26, 1996.

BACKGROUND. The clinical value of DNA ploidy analysis in prostate carcinoma has been an issue for investigation for more than 2 decades. In general, diploid or pseudodiploid tumors are associated with a favorable prognosis and aneuploid tumors with an unfavorable prognosis, irrespective of type of treatment. Tumors with DNA values in the tetraploid region (around 4c) present a diagnostic problem. Such DNA distributions may clearly represent aneuploid tumors with an unfavorable prognosis. However, a 4c distribution may conversely represent a tetraploid tumor (possibly a polyploid variant of the diploid tumor) with a favorable prognosis. Previous data from our laboratory indicate the existence of such a tetraploid subgroup. The goal of the current study was to investigate the diagnostic problem of 4c tumors in greater detail.

METHODS. Ploidy classification of cytologic smears by image cytometry was performed in a retrospective study of 334 patients with hormonally treated prostate carcinoma. Follow-up time was 30 years or until death.

RESULTS. Three ploidy types were defined: near-diploid (D type), near-tetraploid (T type), and highly aneuploid (A type). Tumors with a modal value within the tetraploid region were found in 27% (92 cases) of the total material. Of these, 9% were defined as T type and 18% as A type. Overall, 37% of the tumors were classified as D type, 9% as T type, and 54% as A type. Of the A type tumors, one-third had modal DNA values in the tetraploid (4c) region. Multivariate analysis showed a statistically significant difference between A type tumors and D and T type, but not between D type and T type. Both D and T type tumors progressed slowly and killed the patients 5 to 30 years after diagnosis, whereas A type tumors progressed rapidly and killed the patients within 6 years of diagnosis.

CONCLUSIONS. By image cytometry, prostate carcinoma can be divided into three ploidy types: D, T, and A type. Biologically, however, the tumors fall into only two groups: low grade malignant, pseudodiploid tumors of D or T type, and high grade malignant, highly aneuploid tumors of A type. ***Cancer* 1996; 78:1748–55.**
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KEYWORDS: prostate carcinoma, image cytometry, DNA ploidy, tetraploid tumors, hormonal treatment, prognosis.

Previous studies have shown that May-Grünwald-Giemsa (MGG)-stained cytologic slides from fine-needle aspiration (FNA) biopsies of prostate carcinomas, stored in our files for more than 30 years, can be destained and subsequently Feulgen stained for quantitative image cytophotometric DNA determinations of ploidy level. This procedure allows retrospective studies in which the cytophotometric DNA data can be directly related to the known clinical course of the tumor disease.[1–4]

In our earliest quantitative cytophotometric DNA analyses of hormonally treated prostate carcinoma, the frequency of cells with elevated DNA values (above 2.5c) was used as a gross measure of the degree of aneuploidy. Diploid (or near-diploid) tumors thereby were

distinguished from the highly aneuploid tumors. We found that tumors with diploid (or near-diploid) DNA contents had a considerably less malignant clinical course than tumors with increased (more than 2.5c), clearly aneuploid DNA values.[2,5]

However, in subsequent studies of the clinical course, we found a subgroup of tumors among the tumors with increased DNA values (> 2.5c), which had a much more favorable clinical course than the rest of the tumors. In these tumors, the majority of the cells had DNA values within the tetraploid (4c) region and showed survival times of 15 years or more, similar to that of the diploid (or near-diploid) tumors.[6] It therefore became of interest to identify this "tetraploid" subgroup of prostate carcinoma in more detail. Limits for the diploid (2c), as well as the tetraploid (4c) region were determined. By calculating the percentage of cells with DNA values outside these regions and combining this information with the modal value of the tumor cell population, a ploidy classification was defined by which prostatic tumors could statistically be divided into three ploidy types. The tumors were classified as diploid or near-diploid (D type), tetraploid or near-tetraploid (T type), and highly aneuploid (A type).[6] In our nonselected retrospective clinical material of hormonally treated prostate carcinoma, approximately 50% of the tumors were found to be of A type, 40% of D type, and 10% of T type, at the time of diagnosis.[4]

In studies using flow cytometry, tetraploid tumors are difficult to define and therefore not easily distinguished from the aneuploid tumors, especially those with a modal value within the tetraploid region. To define tetraploidy versus aneuploidy by flow cytometry, different statistical techniques have been recommended, but it is still a matter of controversy. Therefore, in most published flow cytometric studies of prostate carcinoma, a distinction between tetraploid and aneuploid tumors is not made.[7–11] The most common procedure is to define tumors as diploid and nondiploid. In this way, tetraploid tumors are recorded as highly malignant aneuploid tumors. However, flow cytometric studies in which a distinction between tetraploid and aneuploid tumors is made indicate that tetraploid tumors, clinical stage T2, (treated by prostatectomy) are associated with a favorable prognosis[12] in line with our previous studies.[4–6]

The goal of the current study was to elucidate the methodologic aspects of defining "tetraploid" prostate tumors and to analyze the clinical course of these tumors in detail. The study was performed using a large retrospective material of hormonally treated patients, with a long follow-up time (30 years or until death). T type tumors, as defined in the current study, represent approximately 10% of prostate carcinoma and show a clinical course as favorable as that of the diploid, D type tumors. The Cox multivariate analysis showed that there is a significant difference in the survival rate between A type tumors and D and T type tumors, but no difference was found between T type and D type tumors. Furthermore, T and D type patterns often coexist in the same tumor, indicating that T and D type represent two ploidy variants of the same tumor type.

MATERIALS AND METHODS

Clinical Material

The patient material in the current study was from 334 patients with prostate carcinoma. Diagnosis was performed on cytologic slides obtained by FNA biopsy at the Karolinska Hospital, and the patients were registered in the Swedish Cancer Registry during the time period 1960–1969. Only patients who died of prostate carcinoma or survived more than 15 years from diagnosis, irrespective of cause of death, were included in the study.

From a total number of 486 patients, 152 were excluded. In 31 cases the diagnostic slides were not suitable for DNA analysis, and in 121 the diagnostic slides and/or the clinical records or autopsy protocols could not be found, leaving 334 patients for analysis.

The distribution of the 334 patients with respect to ploidy, grade, and TNM classification is shown in Table 1. In the total material, 269 patients died of prostate carcinoma, 54 died of intercurrent disease at least 15 years after diagnosis, and 11 were still alive at the last complete statement of the Cancer Registry on May 1, 1995. The follow-up time was 30 years or until death. The mean age at the time of diagnosis was 67.2 years.

Each of the 334 patients initially or at sign of tumor progression received estrogen therapy and/or bilateral orchiectomy. For 54 patients, radiation therapy was given as palliative treatment. None of the patients were treated surgically with radical prostatectomy.

Cytologic grading was performed in 332 cases. The smears were graded according to a system described previously.[13] Clinical stage was classified according to the system of the International Union Against Cancer with respect to T (size of primary tumor), M (distant metastases), and N (regional lymph node metastases) using information from the cytologic and/or clinical record.[14] In 15 cases, tumor size was not reported. Distant metastases were reported in 19 cases and 4 cases had reported regional lymph node metastases at the time of diagnosis (Table 1).

Cytochemical Procedures

The original MGG-stained routine cytologic smears, on which the primary diagnoses of prostate carcinoma were based, were used for the cytophotometric DNA

TABLE 1
Composition of 334 Cases Analyzed with Respect to Ploidy and Grade, Tumor Size (T2, T3, T4), Distant Metastases (M1), and Regional Lymph Node Metastases (N1) at the Time of Diagnosis and Status

	D type (n = 123)	T type (n = 31)	A type (n = 180)
Grade 1 (n = 82)	51	11	20
Grade 2 (n = 165)	62	15	88
Grade 3 (n = 85)	10	5	70
Not determined	—	—	2
T2	107	23	107
T3	11	7	47
T4	1	—	16
Not determined	4	1	10
M1	1	—	18
N1	—	—	4
Status			
Alive	10	1	—
Dead of cancer	71	18	180
Dead of intercurrent disease	42	12	—

D type: near diploid; T type: near tetradiploid; A type: highly aneuploid.

analysis. One smear per case was analyzed. The cytologic sampling procedure used was the standard method for FNA biopsy. To obtain representative tumor material, four to eight passes with the needle were made in different directions in the tumor area. The slides were destained in absolute methanol for 1 to 3 weeks, refixed in 10% neutral buffered formalin for 12 to 24 hours, [2,3] and restained according to a modified Feulgen staining procedure (which includes acid hydrolysis in 5 N hydrochloric acid for 60 minutes at 22 °C).[15,16] The cytophotometric measurements of Feulgen-stained cell nuclei were performed with a photographic densitometer.[17] Tumor cells were selected for photography on the basis of cytologic malignancy characteristics.

Histogram Interpretation

In each slide, 50 to 100 tumor cells were measured to establish a conclusive histogram. For 99% of the cases (i.e., 332 of 334 cases), DNA analysis of 50 tumor cells was sufficient to unambiguously distinguish T type tumors from A type tumors with a modal value within the tetraploid region. In only 2 cases, 1 hundred cells had to be analyzed to obtain a defined histogram.

The use of an internal staining control is an absolute requirement for accurate ploidy determinations of tumor cells. We used granulocytes for this purpose. The median (P_{50}) DNA value of the control cells was calculated to determine the normal diploid (2c) content of DNA. The 2c value was determined from measurements of a minimum of 20 control cells. All measured DNA values of the tumor cells were expressed in c units as defined by the DNA content of the corresponding control cells. To distinguish D type and T type tumor cell populations from A type tumors, the percentage of tumor cells outside the 2c region (< 2.5c) and the 4c region (3.5c to 4.5c) were determined for each slide.[6,18]

The modal value was defined as the most frequent c value, using a class width of 0.5c and an increment of 0.25c.

Definitions of Ploidy Types

D type tumors were defined as tumors in which more than 50% of the tumor cells had DNA values within the diploid and the tetraploid regions and the modal value was within the diploid region.

T type tumors were defined as tumors in which more than 50% of the tumor cells had DNA values within the diploid and the tetraploid regions and the modal value was within the tetraploid region.

A type tumors were defined as tumors in which more than 50% of the tumor cells had DNA values outside the diploid and the tetraploid regions (Fig. 1).

STATISTICS

The Kaplan–Meier method was used to construct life tables of cause specific death intensity (hazard rate).[19] Cox multivariate regression analysis was applied to survival data.[20] The following parameters were considered: age, ploidy (D, T, and A type), grade, and stage (TNM). Student's *t* test was used to test differences between means and the chi-square statistic to test differences in distribution between groups. The correlation between variables was estimated with Pearson product moment correlation coefficients.

RESULTS

Definitions of D, T, and A Type Tumors

DNA distribution patterns obtained from cytophotometric measurements of prostate carcinoma are exemplified in Figure 1. D and T type tumors show a DNA distribution pattern similar to that of benign prostatic lesions (i.e., the majority [more than 50%] of cells have DNA values within the diploid [2c] region and/or the tetraploid [4c] region).[6] The limits for the diploid (2.5c) and tetraploid (3.5c–4.5c) regions are illustrated by the dotted lines in the figure. The tumors are referred to as D type if the modal value is within the diploid region and as T type if the modal value is within the tetraploid region. Conversely, in A type tumors the majority (more than 50%) of the cells have DNA values outside the 2c and 4c regions, and the modal values are highly variable (Fig. 1:3–5). To unambiguously distinguish T type from A type tumors, especially those

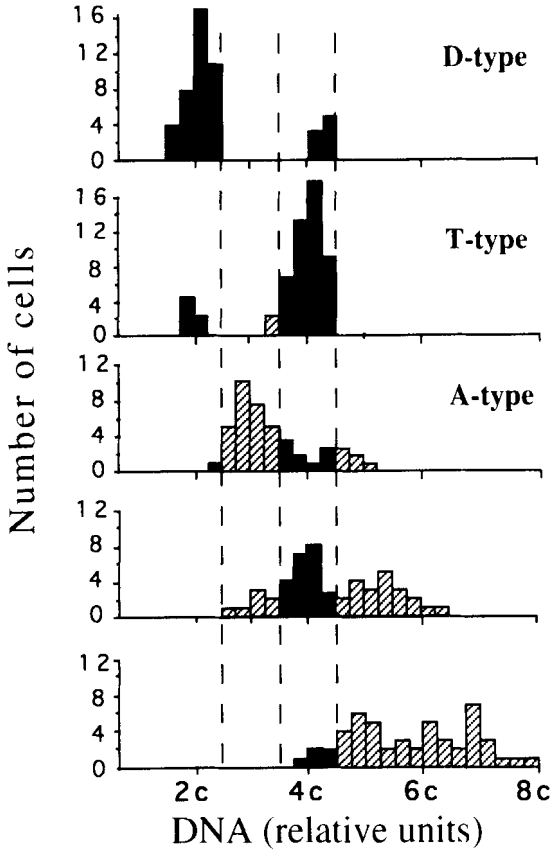

FIGURE 1. DNA distribution in prostate carcinoma. D type (1:1) and T type (1:2) denote predominantly diploid or tetraploid DNA distribution, respectively. A type (1:3–5) denotes a highly aneuploid DNA distribution: A type with modal value within the 3c region (1:3), A type with modal value within the 4c region (1:4), and A type with modal value within the 7c region (1:5). The dotted lines in the histograms denote the upper diploid limit, 2.5c, and the limits of the tetraploid region, 3.5c–4.5c.

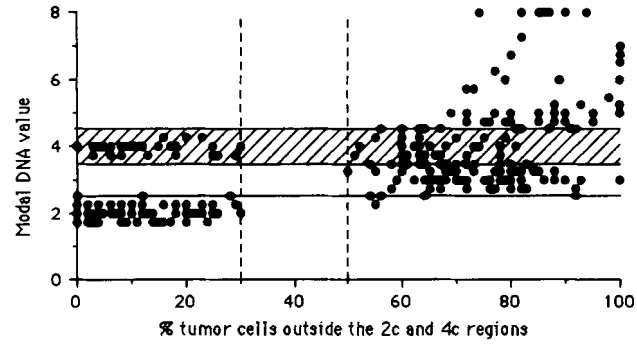

FIGURE 2. Percentage of tumor cells outside the 2c and 4c regions, related to the modal DNA value in c units of the tumor cell population in 334 cases of prostate carcinoma.

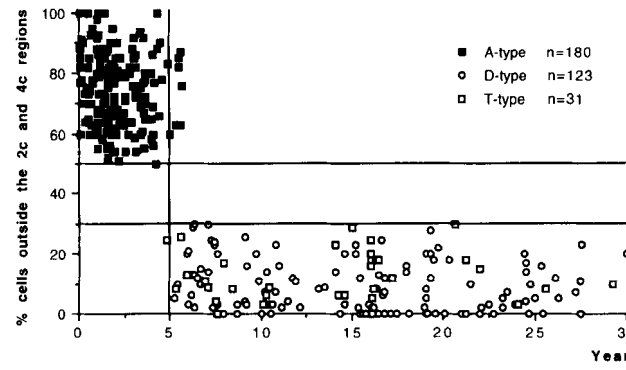

FIGURE 3. Relationship between years of survival from date of diagnosis and percentage of tumor cells outside the 2c and 4c regions in 334 cases of prostate carcinoma.

with a modal value within the tetraploid (4c) region, it is recommended that at least 100 tumor cells be measured.

The result of the cytochemical tumor analyses of the 334 cases is shown in Figure 2. By combining the percentage of tumor cells outside the 2c and 4c regions with the modal value of the tumor cell population, the tumors were clearly divided into three groups, corresponding to the defined three ploidy types. As seen in Figure 2, the defined limits for the 2c and 4c regions manage to separate the D type and T type tumors (Fig. 2, left) from the A type tumors (Fig. 2, right) to such an extent that a gap of 20%, with respect to cells outside the 2c and 4c regions, was obtained. The highest percentage of cells outside the 2c and 4c regions obtained in D and T type tumors was 30% and the lowest percentage in A type tumors was 50%. In addition, Figure 2 shows that some of the A type tumors have a modal value within the 4c region, similar to that of T type tumors. Difficulties may therefore appear in distinguishing these two tumor types from each other on the basis of the modal value alone. However, the determining factor, as described above, is that A type tumors have more than 50% of the tumor cells outside the 2c and 4c region, whereas T type tumors have the majority within the 2c and 4c region. Tumors with a modal value within the 4c region were found in 92 of the 334 cases (28%). Of these, 31 tumors (33%) were classified as T type and 61 (66%) as A type. The overall observed distribution of ploidy was 54% A type (180 of 334 cases), 37% D type (123 of 334), and 9% T type (31 of 334).

Ploidy and Survival

The survival times of the 334 patients with respect to ploidy type are shown in Figure 3. There is a remarkable difference in the clinical course of the D and T type tumors and A type tumors. As many as 97% (174 of 180) of the patients with A type tumors died of cancer during the first 5 years after diagnosis and all

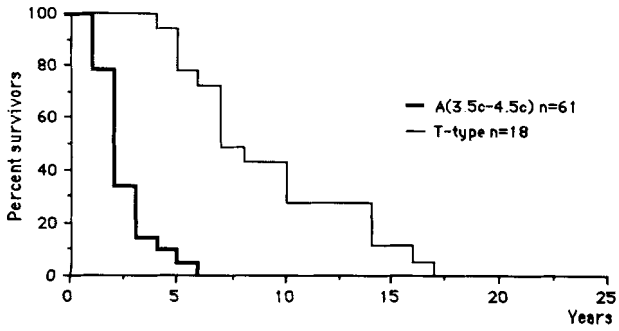

FIGURE 4. Life table of cause specific death intensity (hazard rate) for patients with prostate carcinoma of T type and patients who have A type tumors with a modal value in the tetraploid region (test for heterogenity chi-square = 52; $P < 0.001$).

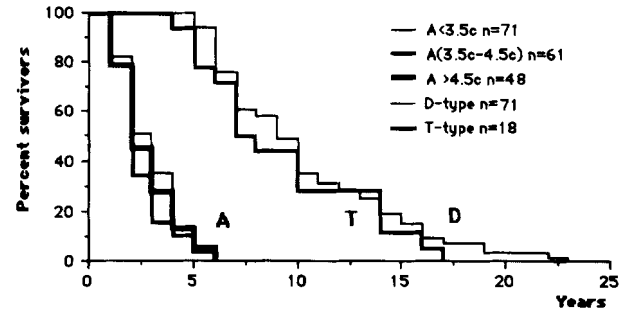

FIGURE 5. Life table of cause specific death intensity (hazard rate) for 269 patients with prostate carcinoma (test for heterogenity chi-square = 249, (df = 4); $P < 0.001$).

patients (100%) had died within 6 years. In contrast, only 1 of the patients with a D or T type tumor died of cancer during the first 5 years after diagnosis, and many of the patients were still alive 15 to 25 years after diagnosis. Furthermore, as Figure 3 shows, there is no obvious difference in the survival time between D type and T type tumors. This was further analyzed in detail.

Survival of Patients with Tumors with a Tetraploid Modal Value

The survival rate for T type tumors was compared with that for the A type tumors with modal values within the 4c region (Fig. 4). In the construction of Kaplan–Meier survival curves for cause specific death intensity, only patients who died of prostate carcinoma were analyzed (i.e., 269 cases). Tumors with a tetraploid modal value were found in 79 of the 269 cases (29%). Eighteen cases were classified as T type and 61 as A type. A strong, highly significant, prognostic difference was found showing that the 18 patients with T type tumors had a much more favorable clinical course than the 61 patients with A type tumors with a modal value within the 4c region (chi-square = 52; $P < 0.001$) (Fig. 4). All the patients with A type tumors had died of cancer 6 years after diagnosis, whereas only 4 of the patients with T type tumors died of cancer during the same time period. Thus, 78% of the patients (14 of 18) with T type tumors were still alive 6 years after diagnosis.

Figure 5 show the survival rate for all 269 patients who died of prostate carcinoma, with respect to ploidy type. The A type tumors are divided, with respect to the modal value obtained, into three groups: A tumors with modal value below, within, or above the 4c region. No significant prognostic difference in the survival rate was found between A type tumors with a modal value in the 4c region and A type tumors in general (with other modal values) (chi-square = 249; degree of freedom (df) = 4, $P < 0.001$) (Fig. 5). Furthermore, the statistical analysis did not show any significant difference in the survival rate between patients with T type tumors and those with D type.

Relation between Ploidy and Grade

The distribution of the 334 patients with respect to ploidy, grade, and TNM classification is shown in Table 1. All ploidy types were represented among all grades (test for heterogeneity, chi-square = 57.4; df = 4; $P < 0.001$). Most Grade 1 tumors (62%; 51 of 82 cases) were of D type, with a favorable prognosis, and most Grade 3 tumors (82%; 70 of 85 cases) were of A type, with a poor prognosis. Approximately half of the tumors were classified as Grade 2. In this group, the D and T type, both with a favorable prognosis, and the A type were approximately equally common. However, the correlation between grade and ploidy was not particularly strong because 15 of the 85 Grade 3 tumors (18%) were of D or T type, and 20 of the 82 Grade 1 tumors (24%) were of A type.

Cox Multivariate Regression Analysis

To further analyze which of the prognostic factors (ploidy, grade, and stage) is the strongest predictor of survival, Cox multivariate regression analysis of disease specific survival was performed (Table 2). Age was included as a control factor. The analysis clearly shows that ploidy (A type versus D and T type) is the strongest predictor of survival (chi-square = 114.6, $P \leq 0.001$) and the factor of paramount importance. Grade contributes additional information to that provided by ploidy only to a small extent (chi-square = 4.6, $P = 0.033$). Grade alone (univariate analysis) proved to be an important predictor of survival in the absence of ploidy (chi-square = 64; $P = 0.001$). In this study, the number of patients with distant metastases at the time of diagnosis, was only 19 (accounting for 6% of the total material), which makes this parameter of limited

TABLE 2
Cox Multivariate Analysis with Respect to Disease Specific Survival: Relative Hazard and 95% Confidence Intervals

Factor	RH	CI	*P* value	Chi-square
Age	1.023	1.009–1.038	0.002	9.9
Ploidy 3 (3 vs. 1,2)	115.1	48.3–274.4	<0.001	114.6
Grade 1 (1 vs. 2,3)	0.697	0.502–0.969	0.033	4.6
Distant metastases	1.742	1.058–2.846	0.028	4.8

RH: relative hazard; CI: 95% confidence interval.
Competing factors were age (continual), ploidy, grade, tumor size (T), distant metastasis (M), and regional lymph node metastasis (N).
Coding: ploidy: D type = 1; T type = 2; A type = 3; Grade: 1, 2, and 3; tumor size: T2 = 1; T3 = 2; and T4 = 3; M: not present = 0; present = 1; N: not present = 0; present = 1; status: alive = 0; dead of cancer = 1; and dead of intercurrent disease = 2.

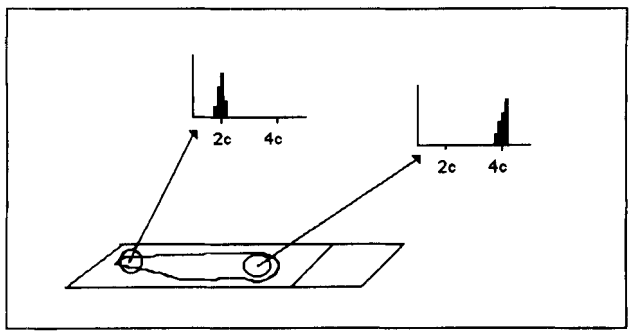

FIGURE 6. Diagram of a slide of a T type prostate carcinoma. Two areas of the tumor as well as their corresponding DNA histograms are shown.

importance. Cox analysis also show that there was a significant difference with respect to survival between A type tumors and D and T type but not between D type and T type tumors.

D and T Type Ploidy Patterns May Coexist in the Same Tumor

A coexistence of diploid and tetraploid DNA patterns is observed in T type tumors as well as in D type tumors, both in cytologic smears and in histopathologic sections of prostate carcinoma. We have found that approximately 10% of the prostate tumors have a predominantly tetraploid DNA distribution. Areas with a diploid DNA distribution can, however, always be found in such tetraploid tumors. This is illustrated in Figure 6. Thus, within the same tumor, one area shows a D type pattern and another area a T type pattern. In the current study, we did not detect the coexistence of an A type pattern within D and T type tumors. However, we cannot exclude the possibility that a patient can have both a D/T type prostate tumor and another, separate prostate tumor of A type.

CONCLUSIONS

In the current study, methodologic and prognostic aspects of tetraploid or near tetraploid prostate tumors were studied. Previous studies have shown that, by the use of image cytometry, it is possible to perform accurate DNA analysis and ploidy level determination of old archival cytologic tumor specimens.[1,6,17] This technique conveniently allows retrospective studies in which, with the use of a very long follow-up time, DNA data can be correlated directly to the known clinical course of the tumor disease.[1,3,4,6,17] Retrospective studies with follow-up times of decades are especially valuable in investigations of tumors with a slow tumor progression rate (i.e., long clinical course).

In our previous retrospective studies, the prostate tumors were cytophotometrically classified into only two tumor types. As a gross parameter of aneuploidy, the percentage of tumor cells exceeding the upper limit of the diploid region (2.5c) was used.[1,2,5,17] Tumors with a diploid or near-diploid DNA content thereby were defined and distinguished from tumors with an increased (more than 2.5c) DNA content. However, the prognostic analysis of these two tumor types showed that diploid tumors had a favorable prognosis but not all of the tumors with increased DNA values had an unfavorable prognosis. Thus, among the tumors with increased DNA values was a group of tumors with a much more favorable clinical course.[1,2,6] In common for these tumors, with increased DNA values and a favorable prognosis, was a predominantly tetraploid or near tetraploid DNA distribution. Therefore, in addition to the limit for the diploid region (2c), limits for the tetraploid region (4c) had to be determined to classify ploidy of prostate carcinoma accurately.

This study confirms that among the aneuploid, nondiploid, tumors is a group of tumors that have a much more favorable clinical course than the rest of the highly aneuploid, A type tumors. Using the method described here, these tumors are defined as T type. Of the patients who died of prostate carcinoma, tumors with a tetraploid DNA distribution were found in 29% (79 of 269). Of these tumors, the majority (61; 22%) were A type and 18 (7%) T type. Kaplan–Meier curves of cause specific death intensity showed that A type tumors with a modal value within the tetraploid region progressed rapidly and killed 97% of all patients within 5 years and all of the patients within 6 years. This progression rate did not differ statistically from the rest of the A type tumors, irrespective of modal value. Conversely, the T type tumors had a progression rate similar to that of the D type. Only 1 patient with a T type tumor died of cancer within the first 5 years after diagnosis and many of the D type patients were still alive after 15 to 25 years. This difference in the survival

rate between the highly malignant A type tumors (irrespective of modal values) and the low malignant D and T type was statistically highly significant.

This result was confirmed by the Cox multivariate analysis of survival, which showed a strong statistically significant difference between A type tumors and D and T type tumors, but not between D type and T type tumors. Thus, the analyses clearly indicate that D and T type tumors should be regarded as one tumor type, DT type. Whether the difference in the clinical course between A type and the D and T type is an effect of the endocrine therapy or a result of the inherently low degree of biologic malignancy among the D and T type tumors remains to be investigated.

Further evidence for the existence of a DT type is the coexistence of D and T type areas within the same tumor. Thus, in T type tumors areas of D type pattern were found and vice versa. However, we have not detected the coexistence of A type patterns and D and T type patterns within the same tumor. We do not consider the coexistence of D and T type as being an expression of genetic heterogeneity. Rather, it indicates that the D type and the T type patterns represent two different states of the tumor and should be regarded as an expression of polyploidization, or alternately that the T type pattern represents a part of the tumor in which many cells are arrested in the G_2 phase.

Cox multivariate regression analysis also showed that ploidy, defined as A type versus D and T type, is the paramount single predictor (chi-square = 114.6) of survival in comparison with grade and stage, and that grade contributes only a small amount of additional information (chi-square = 4.6). However, in the absence of ploidy, grade alone is an important predictor of survival (univariate chi-square = 64).

In most studies using flow cytometry, no distinction between aneuploidy and tetraploidy is made, mainly due to difficulties in establishing a correct definition of tetraploidy.[7–11] The most common procedure is to define tumors as diploid and nondiploid. Tetraploid tumors thereby are recorded as highly malignant aneuploid tumors. The published literature on different methodologic aspects of ploidy determinations has recently been critically evaluated.[21]

Flow studies in which a distinction between different types of nondiploid tumors is made indicate that tetraploid tumors, clinical stage T2 (treated by prostatectomy), in line with our results, have a favorable prognosis similar to that of diploid tumors.[12] However, in more advanced clinical stage D1 diseases (treated by prostatectomy) the tetraploid tumors have a much more malignant clinical course than the diploid tumors, similar to that of aneuploid tumors.[22] In both these studies (clinical stage B and D1 tumors), the DNA ploidy determinations are performed by flow cytometry, but the analyzed tumor cell nuclei are from a suspension of nuclei extracted from thick cut sections of formalin fixed, paraffin embedded tissue blocks.[23] However, this method has been shown to entail several artefacts produced for example by selective destruction of large, fragile aneuploid nuclei, uncontrollable admixture of normal cells, and a variable stainability within the same preparation.[24] This can explain why in such a progressive tumor disease as D1, as many as 46% of the tumors were classified as tetraploid and only 17% as aneuploid.[22] Thus, the majority of the tumors classified by flow cytometry as tetraploid might in fact be aneuploid tumors. Conversely, in clinical stage B tumors one does not expect to find a high percentage of aneuploid tumors and the content of damaged aneuploid cell nuclei should thus be low. In the study of clinical stage B tumors,[12] as expected, only 4% of the tumors were classified as aneuploid, 28% as tetraploid, and 68% as diploid. Thus, the contradictory prognostic results in these two studies of clinical stage B and D1 tumors illustrate the difficulties of using a cytometric method in which unidentified cells are measured, rather than indicating that tetraploid tumors have an unfavorable prognosis in high stage tumor diseases.

Ploidy analysis by image cytometry can also be determined with an acceptable degree of accuracy using histopathologic sections from prostatic tumor material.[1,17] D type tumors thereby are easily distinguished from A type tumors. However, some problems can occur using histopathologic sections in distinguishing T type tumors from A type tumors with a modal value within the 4c region. However, cytologic material is ideal for DNA ploidy determinations by image cytometry because whole and intact nuclei are analyzed, which is not the case with sections. It would therefore be very beneficial if cytologic material was routinely obtained, even in clinics that use core needle biopsy or surgery for diagnosis. Analysis of this material by image cytometry would provide valuable supplementary prognostic information.

In conclusion, in prostate carcinoma, approximately 30% of the tumors have a tetraploid DNA distribution. Using the method described here, however, one-third of these were defined as T type and the rest as A type. In the hormonally treated patients examined in this study, T type tumors have a clinical course as favorable as D type tumors. Thus, prognostically there is no need to distinguish T type tumors from D type tumors. T and D type patterns coexist within the same tumor area, which furthermore demonstrates that the D and T type ought to be regarded as one tumor type, DT type. A type tumors, irrespective of DNA distribution and location of modal value, are a highly malignant form of prostate carcinoma, in contrast to the

low malignant DT tumors. A type tumors progress rapidly and kill the patients within 3 to 5 years. Progression also takes place in DT type tumors with development of metastases and eventually death from cancer in some of the patients (i.e., those who do not die from intercurrent diseases). This process takes 10 to 25 years; it is 3–5 times slower in the DT type tumors than in the A type. The genetic background to the difference in the progression rate between D and T type tumors and A type is unclear. It might reflect a higher degree of genomic instability, leading to an increased mutational frequency, in the A type tumors than in the D and T type. In breast carcinoma, for example, gene amplification, as one marker of genetic instability, is significantly more common among A type than DT type tumors.[25] A similar situation may well prevail in prostate carcinoma. We have also observed that most patients who are diagnosed as having DT type tumors and who eventually die from cancer after several decades still have DT type tumors at the time of death (unpublished data); conversion from DT type to A type appears to be a rare event in hormonally treated patients with prostate carcinoma.

REFERENCES

1. Forsslund G, Zetterberg A. A quantitative evaluation of cytophotometric DNA-analysis in retrospective studies using archival tumor specimens. *Anal Quant Cytol Histol* 1990; 12:259–66.
2. Zetterberg A, Esposti PL. Prognostic significance of nuclear DNA level in prostatic carcinoma. *Scand J Urol Nephrol* 1980;55:53–8.
3. Eneroth CM, Zetterberg A. The relationship between the nuclear DNA content in smears of aspirates and the prognosis of mucoepidermoid carcinoma. *Acta Otolaryngol (Stockh)* 1975;80:429–33.
4. Forsslund G, Esposti P, Nilsson B, Zetterberg A. The prognostic significance of nuclear DNA content in prostatic carcinoma. *Cancer* 1992;69(6):1432–9.
5. Zetterberg A, Esposti PL. Cytophotometric DNA-analysis of aspirated cells from prostatic carcinoma. *Acta Cytol* 1976; 20:46–57.
6. Forsslund G, Zetterberg A. Ploidy level determinations in high-grade and low-grade malignant variants of prostatic carcinoma. *Cancer Res* 1990;50(14):4281–5.
7. Fordham MV, Burdge AH, Matthews J, Williams G, Cooke T. Prostatic carcinoma cell DNA content measured by flow cytometry and its relation to clinical outcome. *Br J Surg* 1986;73:400–3.
8. Haugen OA, Mjolnerod O. DNA ploidy as a prognostic factor in prostatic carcinoma. *Int J Cancer* 1991;45:224–8.
9. Humphrey PA, Walther PJ, Currin SM, Vollmer RT. Histologic grade, DNA ploidy and intraglandular tumor extent as indicators of tumor progression of clinical stage B prostatic carcinoma. A direct comparison. *Am J Surg Pathol* 1991; 15:1165–70.
10. Lee SE, Currin SM, Paulson DF, Walther PJ. Flow cytometric determination of ploidy in prostatic carcinoma: a comparison with seminal vesicle involvement and histopathological grading as a predictor of clinical recurrence. *J Urol* 1988;140:769–74.
11. Mohler JL, Platin AW, Epstein JI, Becker RL, Michael UV, Sesterhenn IA, et al. Prediction of prognosis in untreated stage A2 prostatic carcinoma. *Cancer* 1992;69:511–9.
12. Montgomery BT, Nativ O, Blute ML, Farrow GM, Myers RP, Zincke H, et al. Stage B prostate adenocarcinoma. Flow cytometric nuclear DNA ploidy analysis. *Arch Surg* 1990; 125:327–31.
13. Esposti PL. Cytologic malignancy grading of prostatic carcinoma by transrectal aspiration biopsy. *Scand J Urol Nephrol* 1971;5:199–209.
14. Hermanek P, Sobin LH, editors. International Union Against Cancer. TNM Classification of malignant tumours. Berlin: Springer-Verlag, 1987:124–6.
15. Decosse JJ, Elello N. Feulgen hydrolysis: effect of acid and temperature *J Histochem Cytochem* 1966;14:601–4.
16. Eneroth CM, Zetterberg A. Microspectrophotometric DNA analysis of malignant salivary gland tumors. *Acta Otolaryngol (Stockholm)* 1974;77:289–94.
17. Forsslund G, Kreicbergs A, Nilsson B, Zetterberg A. Photographic densitometry for quantitative DNA analysis of cytological and histological pecimens. *Anal Quant Cytol Histol* 1992;153–60.
18. Eneroth CM, Zetterbeg A. A cytochemical method of grading the malignancy of salivary gland tumors preoperatively. *Acta Otolaryngol (Stockholm)* 1976;81:489–95.
19. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958;53:457–81.
20. Cox DR. Regression model and life tables (with discussion). *JR Stat Soc B* 1972;234:187–220.
21. Shankey V, Kallioniemi O, Koslowski J, Lieber M, Mayall B, Miller G, et al. Consensus review of the clinical utility of DNA content cytometry in prostate cancer. *Cytometry* 1993;14:497–500.
22. Zincke H, Bergstralh EJ, Larsson-Keller JJ, Farrow GM, Myers RP, Lieber MM, et al. Stage D1 prostate cancer treated by radical prostatectomy and adjuvant hormonal treatment. Evidence for favorable survival in patients with DNA diploid tumors. *Cancer* 1992;70(Suppl 1):311–23.
23. Hedley DW, Friedlander ML, Taylor IW, Rugg CA, Musgrove EA. Method to analysis of cellular DNA content paraffin-embedded pathological material using flow cytometry. *J Histochem Cytochem* 1983;31:1333–5.
24. Askensten UG, Moberger B, Auer GU. Methodological aspects on cytochemical DNA assessments of adenocarcinoma in the endometrium by means of image and flow cytometry using conventionally formalin fixed and paraffin-embedded specimens. *Archiv für Geschwust forschung* 1990;60:209–16.
25. Latham C, Månér S, Blegen H, Eriksson E, Zickert P, Auer G, et al. Relationship between oncogene amplification, aneuploidy and altered expression of p53 in breast cancer. *Int J Oncol* 1996;8:359–65.