Frequency and Distribution of Numerical Chromosomal Aberrations in Prostatic Cancer

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Prostatic cancer frequently shows striking morphological heterogeneity and multifocal growth. To better understand the relationship between chromosomal changes and pathological characteristics, 31 routinely processed radical prostatectomy specimens were studied for the presence of numerical chromosomal aberrations by in situ hybridization with centromeric nucleic acid probes specific for chromosomes 7, 10, 17, X, and Y. In 24 of the cases preoperative core biopsy specimens were available and were examined with the probe for the X chromosome. In eight of the prostatectomy specimens chromosome numbers consistent with a normal male karyotype were found. Three cases, besides diploid chromosome numbers, showed a focal doubling of hybridization signals, consistent with tetraploidy. The other 20 cases displayed numerical chromosomal aberrations to a various degree. In this group the appearance of numerical chromosomal aberrations often showed considerable local heterogeneity, generally coinciding with morphological dedifferentiation, and was significantly correlated with tumor stage ($P = .0004$) as well as primary ($P = .0068$), worst ($P = .0002$), and combined ($P < .0001$) Gleason grades, total tumor volume ($P = .0448$), and the volume of tumor with Gleason grades 4 or 5 ($P < .0001$). In four of the 24 core biopsy specimens no residual tumor tissue was left for cytogenetic examination. In the remaining 20 biopsy specimens the presence or absence of numerical changes matched the result obtained on the corresponding prostatectomy specimen. We conclude that in prostatic cancer the presence of numerical chromosomal aberrations is associated with advanced disease. Especially in low differentiated tumors local heterogeneity in 2chromosome numbers can be very marked. It is possible to forecast the presence or absence of numerical chromosomal changes on preoperative core b6iopsy specimens. HUM PATHOL 25:476–484.

Prostatic cancer, outranked only by lung cancer, is currently the second leading cause of death from malignancy in American and European men.[1] Because of advances in detection the number of cases now being reported has sharply increased. Today, assessment of the malignant potential of prostatic cancer is mainly based on morphological grading, staging, and evaluation of tumor volume.[2] Although significant differences in tumor behavior can be shown between groups of patients for these various factors, their reliability as predictors of the course of any individual tumor is limited. Identification of markers that assist in forecasting the behavior of a specific patient's tumor would be very useful.

Quantitative DNA analysis by flow or image cytometry has been reported to offer additional prognostic information for this disease,[3-5] but small genomic changes are beyond the resolving limits of these techniques. Studies of banded metaphase chromosomes can define subtle genetic changes more precisely, but as in other solid epithelial tumors detection of chromosomal aberrations by karyotyping is restricted by low mitotic activity, poor banding quality, and the small number of cells that can be analyzed. Furthermore, correlation of cytogenetic results with histological features is difficult. The latter point seems to be most critical for prostatic carcinoma with its frequently marked local differences in morphological differentiation. Interphase cytogenetics[6] is not prone to these drawbacks and information about numerical and, to a limited degree, structural chromosomal aberrations can be obtained even from tissue sections routinely embedded in paraffin.[7] Therefore, this method allows screening of a large number of tumor cells, enables correlation of cytogenetic with morphological information, and provides the means to detect focal differences in chromosome numbers within one tumor.

In prostatic cancer numerical and structural aberrations of chromosomes 7, 10, and Y have been described by metaphase cytogenetics.[8-11] Chromosome 17, carrying tumor-suppressor and oncogenes,[12,13] has been implicated in the pathogenesis of other epithelial tumors,[14,15] and the X chromosome harbors the androgen-receptor gene. We focused on these chromosomes to examine the presence and distribution of numerical chromosomal changes in tissue sections of radical prostatectomy specimens and matched the results with characteristics deferred from conventional pathological studies (ie, tumor stage, grade, and volume). We also probed the possibility of detecting numerical chromosomal changes in corresponding preoperative core biopsy specimens.

MATERIALS AND METHODS

Tissues

Prostates from 31 consecutive radical retropubic prostatectomies were examined for this study. Intraoperative frozen sections of obturatorial lymph nodes were negative for metastasis, whereas permanent sections showed nodal dissemination of tumor cells in one case. The patients' ages ranged from 52 to 72 years (mean, 64 years). None had received endocrine treatment. The prostates were inked over their entire surface and fixed in unbuffered formalin for 24 to 72 hours. Accord-

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ing to the Stanford protocol,[16] the seminal vesicles, apex, and base were amputated and the remainder was serially blocked at 3-mm intervals along transversal planes parallel to the initial apical and basal sections (ie, perpendicular to the rectal surface). Histological grading was performed according to the Gleason system.[17] Besides routine light microscopy on hematoxylin-eosin–stained sections, the tumor volume was assessed with a digitizing tablet (HDG 1212, Hitachi, Tokyo, Japan) connected to an 80386 DX personal computer running at 40 MHz. The scanning software was SigmaScan version 3.9 (Jandel Scientific, Corte Madera, CA). The volume of high-grade tumor (Gleason grades 4 and 5) was measured separately with this procedure when distinct high-grade tumor areas were present and was estimated when high-grade tumor was diffusely mixed with lower-grade tumor parts. From each of 24 of the patients seven preoperative core biopsy specimens (six biopsy specimens collected at random from both sides of the apex, middle portion, and base as well as one biopsy specimen from the palpatory suspect area) were available and were examined by light microscopy (six step sections from each biopsy specimen) and by interphase cytogenetics (two biopsy specimens with the worst Gleason grade and the largest tumor infiltration). Biopsies were performed with 18-gauge "Tru-cut" biopsy needles (Travenol, purchased by Bard Urological, Covington, GA) driven by the spring-loaded Biopty gun (Bard Urological, Covington, GA). For staging the second revision of the fourth edition of the International Union Against Cancer (UICC) classification system was used.[18]

DNA Probes

The following biotinylated nucleic acid probes of the alphoid-satellite family were used for the prostatectomy specimens: chromosome 7 (D7Z1), 10 (D10Z1), 17 (D17Z1), X (DXZ1), and Y (DYZ3) (Oncor, Gaithersburg, MD). Hybridizations on sections of the core biopsy specimens were performed with the probe for chromosome X only.

Interphase Cytogenetics

Paraffin sections (6 μm) were adhered to silanized glass slides and air dried at 60°C.[19] Sections were dewaxed in xylene (2 × 10 minutes), rinsed in methanol (2 × 5 minutes), and air dried. Subsequently, sections were placed in a plastic coplin jar filled with 10 mmol/L citric acid monohydrate, pH 6, and then exposed to microwaves (720 W) in a household microwave oven (EMM 2300-55/1, Electrolux, Lembeek, Belgium) operating at a frequency of 2,450 MHz and with a maximum power output of 800 W with 10 power level settings for 1 minute (time measured after reaching the boiling point).[20] This was followed by treatment with 1 mol/L sodium thiocyanate for 10 minutes at 80°C.[21] After washing with H_2O, the sections were digested with pepsin (Sigma, München, Germany; 2 or 4 mg/mL in 0.2 N HCl) at 37°C for 3 to 15 minutes. After two washes for 5 minutes each in H_2O, the slides were air dried and heated on a heating plate for 30 minutes at 80°C. Each section was covered with the following freshly prepared hybridization solution: 65% deionized formamide, double-concentrated citrate-buffered saline (2× SSC: 0.3 mol/L NaCl, 0.03 mol/L sodium citrate), 10% dextran sulfate, 1 μg/μL salmon sperm DNA, and 0.1 ng/μL biotinylated probe DNA. Sections were covered with cover slides and, after being sealed with rubber cement, were denatured by heating in a 78°C water bath for 10 minutes followed by hybridization at 37°C overnight.

After hybridization coverslips were carefully removed by floating the slides in 2× SSC, followed by two 10-minute washes in 2× SSC, 50% formamide at 40°C or 45°C, and three changes of phosphate-buffered saline (PBS) (10.4 mmol/L Na_2HPO_4, 3.16 mmol/L KH_2PO_4, 150 mmol/L NaCl, and pH 7.6).

Labeled DNA was detected as follows. The slides were incubated with PBS containing 1.5% normal horse serum for 10 minutes at 37°C. The fluid was decanted and a monoclonal mouse anti-biotin antibody (Boehringer, Mannheim, Germany) diluted 1:100 in PBS was added for 30 minutes at 37°C. After three washes in PBS, the slides were incubated for 15 minutes at 37°C with a biotinylated goat anti-mouse antibody (Jackson Immuno Research, West Grove, PA) diluted 1:200 in PBS. After three washes in PBS, sections were covered with peroxidase-conjugated streptavidin (Dianova, Hamburg, Germany) (1 μg/mL in PBS) for 30 minutes at 37°C. After meticulous washing in PBS, diaminobenzidine (Sigma; 0.5 mg/mL in 0.05 mol/L Tris-HCl, pH 7.6 + 0.03‰ H_2O_2) was added for the color reaction. The slides were counterstained with hemalaun, dehydrated in a series of alcohol followed by xylene, and permanently mounted in Eukitt (Kindler, Freiburg, Germany).

Evaluation

In preliminary experiments the hybridization efficiency of every probe under varying conditions of fixation had been tested on prostatic and several other tissues. Slides were evaluated according to accepted criteria.[22] In short, only section areas with hybridization signals in at least 80% of cells were evaluated. Here, the number of signals in 150 to 450 nonoverlapping nuclei was counted. Only signals having approximately the same size were scored (neglecting minor binding sites). Paired spots (split spots) were counted as one signal. In every section plausibility of results was checked by evaluating non-neoplastic tissues (hyperplastic epithelial and stroma cells). The distribution of counted hybridization signals was analyzed graphically and the significance of apparent differences was checked by statistical tests as described below.

Data Analysis

Significance of differences between groups with nonparametric distribution of data was analyzed with the Mann-Whitney test. Multiple comparisons were performed by analysis of variance for nonparametric data (Kruskal-Wallis test with χ^2 approximate P), followed by Dunn's post-test. Two-by-two contingency tables were analyzed with Fisher's exact test. All P values given are two-tailed. $P < .05$ was considered to reflect significant differences between groups. Commercially available software (InStat version 2.02, GraphPad, San Diego, CA) was used for the calculations.

RESULTS

Pathological Findings

The pathological examination of the prostatectomy specimens showed that 12 of the 31 tumors were still in TNM stage II. One case showed infiltration of the bladder neck and therefore had to be classified as pT4a. The remaining 18 cases were pT3 tumors, of which eight infiltrated the seminal vesicle(s) (pT3c). One of the tumors was a transition zone cancer and was still confined to the prostate in spite of a large tumor volume (37.0 cm^3). In one case paraffin sections showed metastasis to regional lymph nodes although the intraoperative frozen sections had been negative. Positive surgical margins were diagnosed in 10 cases.

Interphase Cytogenetics

In stromal cells and nuclei of hyperplastic or atrophic epithelium no clonal numerical deviations were found (Fig 1). In eight of the 31 cases only chromosome numbers consistent with a normal male karyotype were found (Fig 2). Three cases, along with diploid chromosome numbers, showed a focal doubling of hybridization signals, compatible with tetraploidy (for the sake of simplicity the following tumors with chromosome numbers consistent with diploidy and tetraploidy will be called diploid and tetraploid, respectively). The other 20 cases displayed aneuploid numbers for at least one chromosome (Figs 3 and 4). In this group focal differences in chromosome counts were often striking and paralleled the morphological heterogeneity typical for locally advanced prostatic cancer. The evaluation of the hybridization experiments is exemplified in Fig 4, whereas Table 1 gives a summary of the highest signal numbers found in at least 20% of tumor cells. No characteristic chromosomal abnormality could be detected.

The covariation between the chromosome status and the tumor stage was highly significant when tumors were ranked according to apparent diploidy, tetraploidy, and aneuploidy ($P = .0004$, Mann-Whitney test). The same was true when the primary ($P = .0068$), worst ($P = .0002$), and combined ($P < .0001$) Gleason grades were compared between the diploid and the aneuploid groups (Mann-Whitney test; Table 2). In most cases numerical chromosomal aberrations began to appear when the tubular and acinar patterns of Gleason grade 3 were replaced by cribriform proliferations or when infiltrating cords or chains of tumor cells (corresponding to Gleason grade 4) were present. This resulted in marked differences in the mean volume of low differentiated tumor (Gleason grades 4 and 5): 0.1 cm^3 for the diploid group and 5.6 cm^3 for the aneuploid group ($P < .0001$). Because of an unusually large transition zone cancer in the diploid group (case no. 6) differences in the total tumor volume were smaller, but still significant ($P = .048$). In the aneuploid group the smallest volume of low differentiated tumor was 0.4 cm^3. This compares to a maximum volume of Gleason grade 4/5 tumor of 0.5 cm^3 in the diploid group. No significant differences for the aforementioned characteristics were found between the diploid and the tetraploid groups. Nine of the 10 cases with positive surgical margins were recruited from the aneuploid tumor group, whereas in the diploid/tetraploid group only the large transition zone cancer (case no. 6) could not be removed completely.

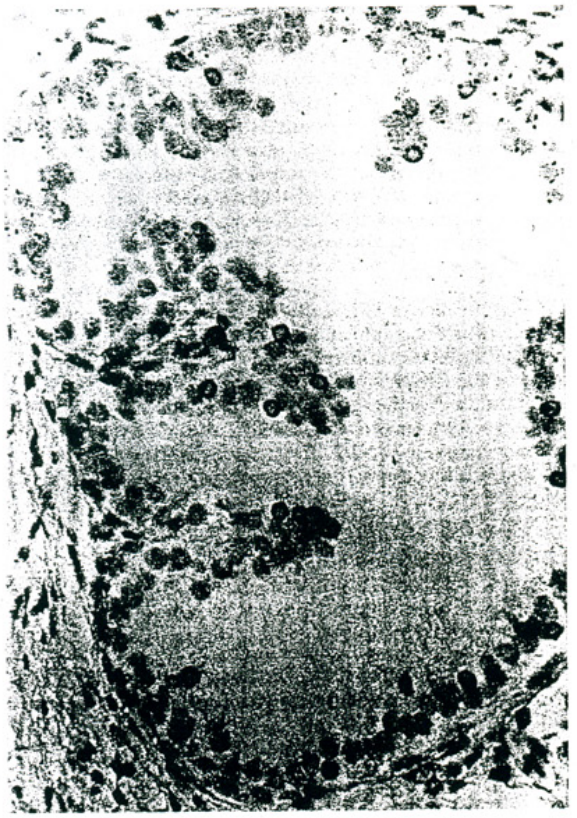

FIGURE 1. Hyperplastic prostatic gland. In situ hybridization with the probe for chromosome X shows a signal distribution that is consistent with a normal monosomy. Only rarely are nuclei with two apparent dots observed. Some of these are artifacts caused by overlapping of nuclei, which is difficult to resolve in one plane of focus. (Original magnification ×400.)

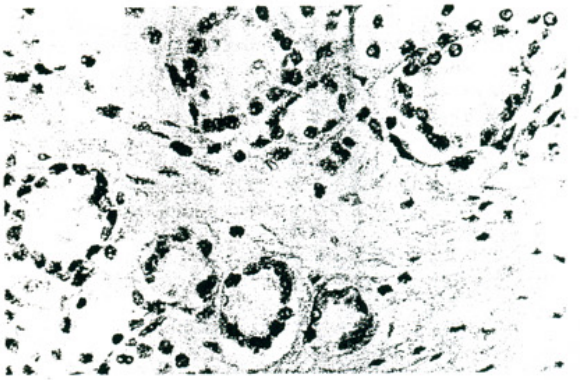

FIGURE 2. Well-differentiated prostatic adenocarcinoma. Hybridization with the probe for chromosome 17 shows two signals in the majority of nuclei, including some stroma cells. There are no cells with more than two dots. Evaluation showed a normal disome distribution. (Original magnification ×570.)

Core Biopsy Specimens

Preoperative core biopsy specimens were available in a total of 24 cases, but in four of these cases no residual tumor was left in the step sections after routine histology had been performed. In the remaining 20 cases hybridization was successful and the number of X chromosomes found was always included in the set of numbers found in the corresponding prostatectomy specimens. In a two-by-two contingency table the correlation of the presence of one or more than one X chromosome in the biopsy specimen and the prostatectomy specimen was significant ($P = .05$, Fisher's exact

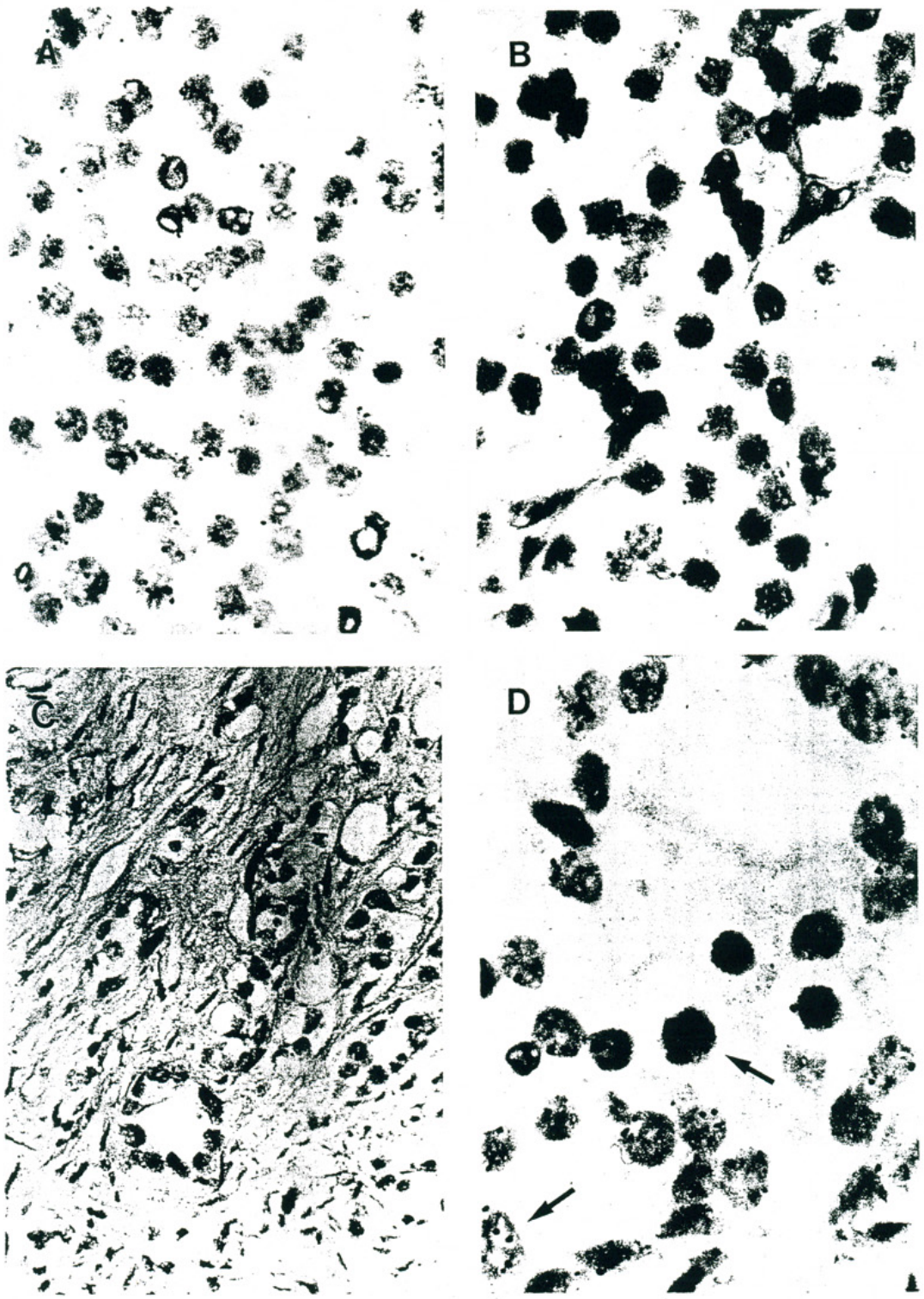

FIGURE 3. Examples of hybridization results in two tumors with local chromosomal heterogeneity. The corresponding evaluation is shown in Fig 4. (A) Case no. 12. Nuclei display a maximum of two signals after in situ hybridization with the probe for chromosome 17. (Original magnification ×1,000.) (B) Same case and probe as in (A). Here, up to four signals are evident. (Original magnification ×1,000.) (C) Case no. 17. In situ hybridization with the probe for chromosome X in an area with recognizable tubular differentiation. Most nuclei show two hybridization signals. (Original magnification ×400.) (D) Same case and probe as in (C). In this cribriform differentiated area of the tumor (Gleason grade 4) most nuclei showed four hybridization signals. Rare cells even show five or six dots (arrows), reflecting chromosomal instability. (Original magnification ×1,000.)

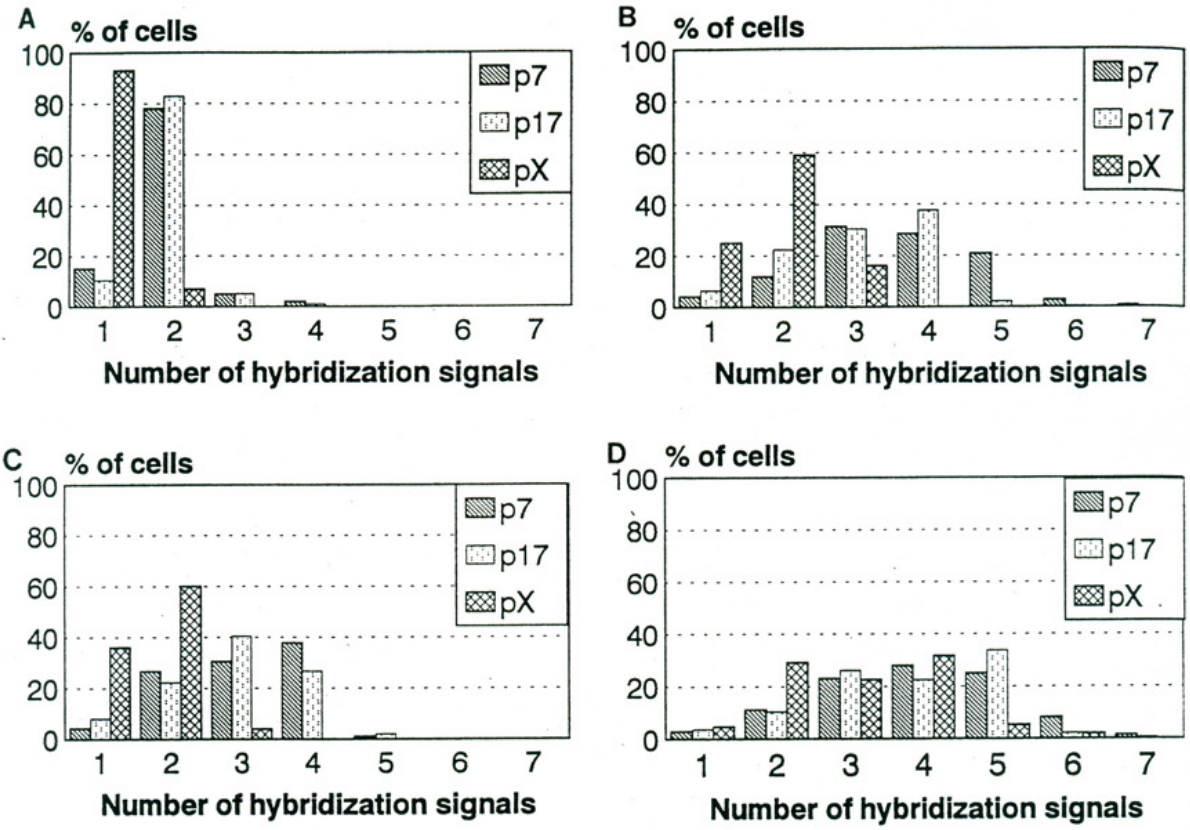

FIGURE 4. Evaluation of in situ hybridization with three chromosome-specific probes, including the experiments shown in Fig 3. Only signal-bearing nuclei were scored. (A) Case no. 12. A normal diploid distribution of signal numbers can be seen. (B) Another area of case no. 12, probed for the same chromosomes as in (A), shows partial aneuploidy. Although the signal distribution of chromosome 17 is still typical for tetrasomy (Fig 3B), chromosome 7 is frankly aberrant with up to five signals in more than 20% of cells. In addition, 16% of the cells display three signals for chromosome X, which is more than would be expected for an unmixed tetraploid population. The *P* for the difference between (A) and (B) is .0000, Kruskal-Wallis test. Comparison of each pair of chromosomes in (A) and (B) by Dunn's post-test showed significant differences ($P < .001$; ie, p7 in (A) versus p7 in (B), and so on). The differences between chromosomes 7 and 17 in a given area (ie, p7 in (A) versus p17 in (A), and so on) are not significant. (C) Case no. 17. In this area the distribution of signal numbers is consistent with tetraploidy (cf. Fig 3C). (D) Case no. 17. High-grade area with aneuploid signal distribution for each of the chromosomes shown here (Fig 3D). The difference in signal counts between (C) and (D) is extremely significant ($P = .0000$, Kruskal-Wallis test). Dunn's multiple comparisons test quotes the differences between individual pairs of chromosomes in (C) and (D) as significant ($P < .001$). The differences between chromosomes 7 and 17 in area (C) or (D) are not significant.

test). Not infrequently, even in these small tissue samples, local heterogeneities in chromosome counts could be noted when tumor glands of different grading were included in one biopsy sample.

DISCUSSION

Interphase cytogenetics on tissue sections offers the possibility to study chromosomal changes of cells embedded in intact histological environs. We applied this method with a set of five chromosome-specific nucleic acid probes on routinely processed tissue sections of prostatic cancer. The data of our study show a close correlation between the appearance of numerical chromosomal aberrations and advanced tumor stages, larger tumor volumes, and a shift of histological differentiation toward higher tumor grades.

Flow and image cytometry are complementary attempts to study the DNA content of prostatic cancer.[3-5] Tribukait, independently confirmed by Koss,[23,24] showed in a series of 500 cases that 80% of small tumors had a diploid DNA content whereas in advanced stages this fraction was reduced to 2%.[25] The first recognized histogram change with increasing tumor grade or stage was tetraploidy. Tribukait[25,26] postulated that as prostatic cancers progress, diploid tumors develop via a tetraploid state to aneuploidy with an estimated transformation rate of 17% and 8.5% per year, respectively. Also, in studies of other solid tumors tetraploidization seemed to be a critical step in tumor progression.[27,28]

The results of our study are consistent with this hypothesized scenario of tumor progression. The data summarized in Table 1 suggest that the characteristics of the

TABLE 1. Summary of Results of Interphase Cytogenetics in 31 Prostatic Carcinomas

Case No.	Age (yr)	PSA (ng/mL)	CB pX	Chromosome Counts* Prostatectomy Specimens p7	p10	p17	pX	pY	Ploidy	Weight (g)	Gleason	Stage	Margin	Volume (cm^3)	Vol-$Gl_{>4}$ (cm^3)
1	67	ni	na	2	2	2	1	1	d	45	2 + 4 = 6	pT2cN0	neg	4.7	0.4
2	64	ni	na	2	2	2	1	1	d	44	3 + 2 = 5	pT2bN0	neg	1.4	0
3	64	7.5	na	2	2	2	1	1	d	60	3 + 2 = 5	pT2aN0	neg	0.3	0
4	62	11.3	nt	2	2	2	1	1	d	49	3 + 2 = 5	pT2cN0	neg	4.1	0
5	52	10.1	na	2	2	2	1	1	d	45	3 + 3 = 6	pT2aN0	neg	4.7	0
6	68	52.7	1	2	2	2	1	1	d	79	3 + 2 = 5	pT3aN0	pos	37.0	0.5
7	61	4.5	nt	2	2	2	1	1	d	60	2 + 3 = 5	pT2cN0	neg	3.6	0
8	72	19.6	na	2	2	2	1	1	d	62	2 + 3 = 5	pT2cN0	neg	0.3	0
9	71	14.1	2	2 (4)	2 (4)	2 (4)	1 (2)	1 (2)	t	40	3 + 3 = 6	pT2cN0	neg	7.2	0
10	54	6.4	2	2 (4)	2 (4)	2 (4)	1 (2)	1 (2)	t	40	3 + 2 = 5	pT2aN0	neg	3.9	0
11	72	15.4	na	4 (2)	4 (2)	4 (2)	2	2 (1)	t	48	3 + 3 = 6	pT2cN0	neg	2.8	0
12	60	72.0	2	5 (2)	2	4 (2)	2 (1)	2 (1)	a	77	4 + 5 = 9	pT4aN1	pos	23.9	23.9
13	64	66.9	2	3	2	3	2	2	a	57	4 + 3 = 7	pT3bN0	neg	12.5	8.8
14	68	6.2	nt	2	2	2 (3)	2	2 (0)	a	52	4 + 3 = 7	pT3bN0	pos	6.7	3.3
15	56	10.1	2	2	3	2	1 (2)	1	a	35	3 + 4 = 7	pT3cN0	neg	11.1	1.1
16	67	7.0	2 (1)	2	2	2 (3)	2 (1)	1	a	42	4 + 3 = 7	pT2bN0	neg	2.9	2.3
17	61	6.9	na	5 (4)	5 (4)	5 (4)	4 (2, 5)	0 (1; 2)	a	64	4 + 5 = 9	pT3cN0	pos	16.4	16.4
18	57	19.7	2	2	3 (2)	4 (2)	2 (1)	2 (1)	a	36	3 + 4 = 7	pT3aN0	neg	4.4	0.5
19	70	13.7	2	4	1 (3)	4 (5)	2 (1)	1 (2, 0)	a	37	4 + 5 = 9	pT3cN0	pos	12.9	10.3
20	45	30.0	2 (1)	2	2	3	2 (1)	1 (2)	a	40	5 + 4 = 9	pT3cN0	pos	22.6	22.6
21	61	6.4	1 (2)	5	4	3	2	2	a	42	3 + 4 = 7	pT3aN0	pos	7.3	0.7
22	53	35.8	3	2 (4)	2 (3)	2 (4)	1 (2, 3)	1	a	33	3 + 4 = 7	pT3aN0	neg	6.7	0.7
23	62	1.5	1 (2)	3	3	2 (1)	1 (2)	2	a	25	4 + 5 = 9	pT3cN0	pos	5.8	5.2
24	56	11.3	1 (2)	2 (3)	2	2	1 (2)	1	a	41	3 + 4 = 7	pT3cN0	neg	4.5	0.4
25	72	10.1	2	2	2 (1)	2	2	2	a	45	3 + 4 = 7	pT3aN0	pos	3.2	0.5
26	66	3.3	2	4	3 (4, 5)	4 (2, 3)	2	2	a	32	5 + 3 = 8	pT3cN0	neg	6.2	4.1
27	54	4.1	2	3 (4)	3 (4, 5)	3	2	2	a	36	3 + 4 = 7	pT3aN0	neg	7.8	0.8
28	69	15.4	2 (1)	2	2	2	1 (2)	1	a	65	3 + 4 = 7	pT3cN0	neg	3.0	0.6
29	64	35.0	2 (3)	2 (3)	2	2 (4)	1 (3)	1 (2)	a	40	3 + 2 = 5	pT2cN0	neg	14.6	2.9
30	64	31.8	2	3	3	4	2	2	a	39	4 + 3 = 7	pT3aN0	neg	6.6	5.0
31	70	3.9	nt	3	3	2	2 (3, 1)	2	a	48	4 + 3 = 7	pT3aN0	pos	2.8	1.5

Abbreviations: PSA, preoperative serum prostate-specific antigen; CB, core biopsy; $Vol_{Gl>4}$, volume of tumor with Gleason grade 4 or 5; ni, no information; na, not available; nt, biopsy with no tumor left after routine histology; d, diploid; t, tetraploid; a, aneuploid; neg, negative; pos, positive.

* Figures indicate the highest number of hybridization signals found in at least 20% of tumor cells. Numbers in brackets denote clonal chromosome counts in minor tumor fractions. In the diploid group (case nos. 1-8) the fraction of nuclei with increased signal numbers did not exceed 5%.

tetraploid tumors are more connected with the diploid than with the aneuploid group. In the aneuploid group there are cases that show a close resemblance to tetraploidy, with aneuploid chromosome numbers disclosed only by some of the five probes. Thus, at least for a part of the tumors in our series, changes of chromosome content according to Tribukait's proposal are quite within the bounds of probability.

The number of tetraploid tumors found in our series is smaller than the frequency reported in studies based on flow cytometry.[25,29,30] This difference might be explained by methodical peculiarities. In interphase cytogenetics on tissue sections numerical changes of a single chromosome can be detected reliably even when they are present only in a small compartment of the tumor, whereas in flow cytometry such subtle changes are likely to be submerged in the noise produced by the dominant tumor fraction. Therefore, tumors that might be judged to be still tetraploid from a DNA histogram could be assigned to the aneuploid group when single chromosomes are counted through the eyepiece of a microscope. In addition, evaluation criteria of tetraploid populations vary between different flow DNA cytometry reports.[31-33] Also, in image DNA cytometry studies different mathematical evaluation criteria for tetraploid populations have been applied.[34-36] Clearly, the advantage of DNA cytometry is that information about the total chromosome content is obtained in a single step, whereas in interphase cytogenetics only spot checks for a limited number of chromosomes can be performed with a reasonable expenditure of energy and time. Thus, these methods complement each other.

Despite the frequency of prostatic cancer in the male population, the number of cases studied cytogenetically has been rather small and chromosomal changes observed in prostatic cancer have so far been difficult to classify into diagnostic and prognostic categories.[37] The chromosomes most often reported to show numerical or structural changes were the Y chromosome, chromosome 7 (7q), and chromosome 10 (10q).[8-11] This was our rationale in including probes for these chromosomes in our study. As can be noted from the gross summary given in Table 1, all these chromosomes showed numerical aberrations in a varying number of cases. However, we found no correlation between particular alterations and certain histopathological characteristics. Loss of the Y chromosome was noted in fewer cases than would be expected from the literature.[8-11] This might have methodical reasons; interphase cytogenetics on tissue sections is certainly more suited

TABLE 2. Comparison of Tumor Characteristics Between Groups With Chromosome Counts Consistent With Diploidy and Aneuploidy

Characteristic	Diploid (N = 8)	Aneuploid (N = 20)	P*
	mean ± standard deviation (median; range)		
Grading (primary Gleason)	2.8 ± 0.5 (3; 2-3)	3.7 ± 0.7 (4; 3-5)	.0068
Grading (worst Gleason)	3.1 ± 0.4 (3; 3-4)	4.3 ± 0.5 (4; 4-5)	.0002
Grading (combined Gleason)	5.2 ± 0.5 (5; 5-6)	7.5 ± 0.9 (7; 7-9)	<.0001
Total tumor volume (cm^3)	7.0 ± 12.3 (3.9; 0.3-37.0)	9.1 ± 6.3 (6.7; 2.8-23.9)	.0448
Volume of tumor with Gleason grade ≥ 4 (cm^3)	0.1 ± 0.2 (0.0; 0.0-0.5)	5.6 ± 7.3 (2.6; 0.4-23.9)	<.0001
PSA (ng/mL)	15.2 ± 13.3 (9.4; 7-35)	33.1 ± 33.3 (13.7; 6.2-72)	.9048
Age (yr)	63.7 ± 5.9 (64; 52-72)	62.0 ± 6.9 (63; 45-72)	.5930

Abbreviation: PSA, preoperative serum level of prostate-specific antigen.
* Mann-Whitney test.

for the detection of chromosomal gains. It can be difficult to distinguish focal insufficient hybridization from chromosomal losses.[38] Therefore, we followed a conservative approach and diagnosed chromosomal loss only when perfectly hybridizing control tissue was in close vicinity to the tumor cells under consideration. This policy may have lowered sensitivity for the detection of chromosomal loss.

Using chromosomal banding Brothman et al[8] found cytogenetically abnormal clonal populations in only five of 20 cases and proposed that early-stage prostatic cancers contain subtle chromosomal changes that escape detection by standard cytogenetic procedures. In another recently published series of 57 tumors[9] clonal karyotypic abnormalities were detected in metaphase spreads of 15 carcinomas, nonclonal aberrations in 18 cases, and normal karyotypes in 24 tumors. Consistent with our results, clonal chromosomal changes were for the most part found in locally advanced or metastatic tumors. This finding also was reported recently by Micale et al[10] who, by karyotyping 62 primary prostatic carcinomas, found clonal aberrations confined to tumors of advanced stage. These investigators suggested that heterogeneity of prostatic cancer in vivo might be the reason for the coexistence of clonal aberrant, nonclonal aberrant, and normal diploid cells in culture. The data of our morphological study document karyotypic heterogeneity in prostatic cancer, emphasizing that it may be very important for cytogeneticists to report precisely on the differentiation of the piece of tumor from which cells for analysis were obtained. For example, results obtained on tissue fragments that were carefully mapped before culturing and karyotyping would be of great interest.

The number of prostatic carcinomas studied by interphase cytogenetics is very limited at the present time. Van Dekken et al[39] examined cytological material from one metastasized tumor and found a diploid pattern for most of the 12 probes they applied. Monosomy of chromosome 10 and loss of the Y chromosome were the only noted deviations. Brothman et al[40] showed aneusomies by fluorescence in situ hybridization with probes for chromosomes 7, 8, 10, 16, 17, and 18 in nine of 10 primary prostatic tumor cultures. There was no correlation of chromosomal loss or gain with Gleason stage or clinical course. These studies identified chromosomal losses more frequently than we did. This is probably due to methodical reasons. Detection of chromosomal loss by interphase cytogenetics is more reliable on cytological material than on paraffin sections, where truncation of nuclei and insufficient hybridization can simulate loss of chromosomes. Therefore, we have been very restrictive in diagnosing chromosomal losses, as mentioned earlier. Van Dekken et al[41] reported on constitutional underrepresentation of the Y chromosome (compared with chromosomes 7 and 16) in tissue sections of one tumor.[41] Recently, we showed by interphase cytogenetics on paraffin sections of 11 prostatic carcinomas that the presence of numerical chromosomal aberrations coincides with immunohistochemically assessed proliferative activity and spotted numerical changes—frequently with marked local heterogeneity—predominantly in advanced cancer.[42] Micale et al[43] compared interphase with metaphase analyses of chromosomes and found significant differences in type and extent of cytogenetic aberrations. These differences were mainly attributed to nonidentity of cell populations studied with each technique. Intratumor heterogeneity of chromosome status has been independently described recently with flow cytometry on a mapped specimen of prostatic cancer.[44]

In our study the occurrence of numerical chromosomal changes correlated well with established determinants of prognosis, such as tumor grade, stage, and the volume of high-grade tumor. This is consistent with data from larger studies of prostatic cancer (by chromosomal banding and DNA cytometry) that reported on an impressive prognostic significance of the appearance of chromosomal aberrations and DNA aneuploidy, respectively.[45,46] The question as to whether interphase cytogenetics can provide additional predictive information beyond that provided by conventional factors alone will be solved after a clinical follow-up of patients who have been carefully matched and controlled for stage, histological grade, and treatment. Next to accuracy, the usefulness of a predictive factor depends on how early in the process of clinical management it is available. While tumor stage and volume can be judged properly only after prostatectomy, interphase cytogenetics, just as flow or image cytometry, can be performed on preoperative core biopsy specimens. Obviously, for a tumor with marked local differences in chromosome numbers one of the main methodical problems is the risk of sampling error. With this difficulty in mind we examined how the detection of numerical aberrations corresponded between biopsy spec-

imens and surgical specimens. We chose the probe for chromosome X for this enquiry mainly for methodical reasons; gains of a chromosome that normally is represented only once in male cells can be detected in a limited number of cells with a higher precision than gains of a chromosome with two regular copies. To increase the probability of identifying pathological numbers of the probed chromosome, we chose the biopsy specimens with the worst Gleason grade from the set of seven that was available from each of 24 patients. Using this approach the normal number or gains of chromosome X could be shown correctly in the biopsy specimens of 20 patients, whereas in the remaining four patients no residual tumor was left after sectioning for routine histology. This problem might be an obstacle predominantly for tumors of better differentiation because these generally are smaller and, as a rule, will occupy smaller fractions of biopsy specimen cores. Nevertheless, our data indicate that the identification of numerical chromosomal aberrations in a biopsy specimen may be an ominous sign. A small fragment of cancer tissue thus may be unmasked as being merely the tip of the neoplastic "iceberg." In combination with all other available data, this information may be helpful for the often difficult decision as to whether a putative small prostatic carcinoma should be treated radically and immediately or whether a more conservative approach is justified.

The present study shows the utility of interphase cytogenetics to assess numerical chromosomal aberrations in tissue sections of prostatectomy and core biopsy specimens. Future studies will show whether the observed close correlation of these changes with conventional prognostic factors will result in the definition of an independent determining factor of clinical course. Cytogeneticists paying attention to and using the marked local heterogeneity of chromosomal deviations in this malignancy are challenged to identify more specific chromosomal changes that mark the transition of slow growing prostatic carcinoma into an aggressive phenotype.

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