Gain and Loss of Chromosomes 1, 7, 8, 10, 18, and Y in 46 Prostate Cancers

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Fluorescence in situ hybridization (FISH) with centromere probes was used to investigate numerical aberrations of chromosomes 1, 7, 8, 10, 18, and Y in 46 prostate carcinoma (PC) and 11 benign prostatic hyperplasia (BPH) samples. None of the benign specimens showed any chromosomal aberration. Forty-one of 46 PC specimens showed numerical aberrations of one or more chromosomes. All investigated chromosomes showed numerical aberrations in at least 30% of the specimens, gain being more frequent than loss. Comparison of DNA flow cytometry (FCM) and FISH results showed that not only aneuploid tumors but also most diploid tumors harbored numerical chromosome aberrations. Chromosome 10 was the most frequently gained (65%), and Y the most frequently lost chromosome (14%). Nonmetastatic and metastatic tumors differed significantly (*P* < .05) in the number of copies for chromosomes 7, 8, and 10, but not for 1, 18, and Y. These results suggest strongly that gains of chromosomes 7, 8, and 10 are involved in PC progression. **HUM PATHOL 27:720–727.** **Copyright © 1996 by W.B. Saunders Company**

***Key words:* fluorescence in situ hybridization, prostatic carcinoma, benign prostatic hyperplasia, DNA flow cytometry, clinical correlation.**

***Abbreviations:* FISH, fluorescence in situ hybridization; PC, prostate carcinoma; BPH, benign prostatic hyperplasia; TUR, transurethral resection; PBS, phosphate-buffered saline; FCM, flow cytometry; ANC, average number of copies; LOH, loss of heterozygosity; LM, lymph node metastasis.**

By cytogenetic analysis, structural and numerical aberrations of chromosomes 1, 7, 8, 10, 16, and Y[1] were identified in about 25% of the prostate carcinomas (PCs) studied. However, this figure is probably an underestimation of the true extent of the aberrations. This is because of selective isolation and preferential in vitro growth of nonmalignant prostate epithelium.[2,3] The use of interphase cytogenetic techniques for characterization of uncultured PC material has been stimulated by these findings. Application of in situ hybridization with centromere-specific DNA probes to fixed sections of PC has shown numerical aberrations for chromosomes 1, 7, 8, 10, 12, 17, 18, X, and Y.[4-7] The finding of numerical aberrations in different chromosomes is not surprising because about 50% of the PCs have an aneuploid DNA content.[8] In the present study, the authors investigated numerical changes of chromosomes 1, 7, 8, 10, 18, and Y using fluorescence in situ hybridization (FISH) with centromere-specific DNA probes on nuclear suspensions of fresh tissue samples from 11 benign prostatic hyperplasia (BPH) and 43 PC patients. Selection of this chromosome panel was based on evidence from the literature and previous studies[9-12] that these chromosomes were possibly implicated in PC development or progression. Study of recurring patterns of specific chromosomal aberrations might provide new information about the genetic events involved in these processes.

The BPH specimens showed no deviation from normal diploidy, so consequently the BPH results were used as a control. Based on this, significant chromosome gains and losses in the PC samples could be analyzed. Possible correlations with clinical parameters and ploidy as determined by DNA flow cytometry (FCM) were investigated.

MATERIALS AND METHODS

Tumor Tissues

Eleven BPH specimens were studied, after being obtained at transurethral resection (TUR) or prostatectomy for BPH. The mean age of the BPH patients was 72.4 ± 5.6 (range, 64 to 80 years). Forty-six PC specimens, from 43 different patients were studied, obtained, and studied at radical prostatectomy (15 specimens), TUR (26 specimens), or pelvic lymph node dissection (five specimens). From one lymph node specimen (PC295), a cell line emerged after xenografting on nude mice. Tissue from this cell line at mouse passage was used instead of the original tissue. The mean age of the PC patients was 66.7 ± 10.5 (range, 49 to 93 years). The percentage of tumor cells present in the tissue specimens was assessed from paraffin sections of adjacent tissue. Only specimens with more than 50% tumor cells were included in the study.

From 23 patients, detailed clinical data could be obtained (Table 1). Twelve patients were lymph node negative (nonmetastatic), and 11 were node positive (metastatic) at the time of first surgery. Three patients had organ-confined disease (T2N0), whereas four tumors showed periprostatic spread (T4). The remaining 16 patients showed extracapsular extension of the tumor (T3N0) or had positive lymph nodes (N+, TNM system for PC 1992[13]). Three of these tumors were well differentiated (G1, all from primary tumors obtained at radical prostatectomy); 10 were moderately differentiated (G2); and in 10, poorly differentiated (G3) areas were found. From three patients (case nos. 4, 5, and 10), two consecutive tumor samples were obtained. The second sample of case no. 4 was obtained from a local recurrence, diagnosed 37 months after radical prostatectomy.

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TABLE 1. Detailed Clinical Data, FCM, and FISH Results From 21 Patients

Case No.	PC NR	Tissue	G	TNM at Surgery	FU*	Status	FCM	Aneusomies†
1	202	TUR	2	T4N3M0	52 (44)	D	A	P1, P7, P8, P10, P18 M1, M7, M8, M10
2	236	LM	3	T3N2M0	50 (0)	DN	D	P10
3	244	LM	2	T2N2M0	56 (0)	D	A	P7, P8, P10, P18
4	256	P	2	T3N1M0	53 (37)	P	T	P8, P10, P18 M8, M10, M18
	384	TUR					T	P1, P7, P8, P10, P18 M7
5	269	LM	3	T3NxM0	15 (0)	D	AH	P1, P7, P8, P10, P18
	291	TUR					A	P1, P8, P10, P18 M10
6	270	LM	3	T0NxM2	17 (0)	D	T	P1, P7, P8, P10, P18, PY
7	288	P	2	T3N0M0	40	NP	D	P1, P8, P10 M8, MY
8	289	P	3	T3N0M0	15 (5)	D	A	P1, P7, P18
9	290	P	2	T3N0M0	38 (31)	P	A	P1, P7, P8, P10, P18 M8, M10, MY
10	295	LM	3	T1NxM0	9 (0)	D	AH	P8, P10 M1, M18, MY
	320	TUR					A	P10 M7, M8, M10, MY
11	296	TUR	3	T4N2M0	13 (0)	P	A	P8, P18 M18
12	341	P	1	T2N0M0	30	NP	D	—
13	342	P	1	T2N0M0	29	NP	A	P1, P7, P8, P10, P18, PY
14	343	P	2	T3N0M0	29	NP	A	—
15	352	P	2	T3N0M0	17	NP	D	M8
16	354	P	3	T3N0M0	26	NP	A	P7, P8, P10, P18
17	362	P	3	T4N0M0	23 (19)	P	A	P1, P8, P18 M18
18	371	TUR	2	T1NxM0	40 (0)	D	A	—
19	382	P	1	T2N0M0	19	NP	D	—
20	389	P	2	T3N0M0	20	NP	D	P7, P10, P18 M7, M10
21	395	P	2	T3N0M0	18	NP	D	M18, MY
22	400	TUR	3	T4NxM2	25 (0)	D	NE	—
23	420	TUR	3	T2NxM0	13 (0)	P	NE	P10 M1, M10

Abbreviations: FISH, fluorescence in situ hybridization; FCM, flow cytometry; PC, prostate carcinoma; P, primary tumor; TUR, transurethral resection; LM, lymph node metastasis; G, tumor grade; Nx, one or more positive lymph nodes; FU, total follow-up time in mo; NP, not progressed; P, progressed; D, deceased from PC; DN, deceased, not from PC; D, diploid; T, tetraploid; A, aneuploid; AH, hypodiploid aneuploid; NE, not evaluable; P, polysomy; M, monosomy or nullisomy.

* Time to progression, when appropriate, in brackets.

† P and M percentages at or above cutoff percentage for each chromosome as specified in Table 2.

Tissue Processing and Sample Preparation

Suspected benign hyperplasia or carcinoma tissues were excised and cut into several smaller fragments, snap frozen in liquid nitrogen, and stored at −80°C. For isolation of cells from tissue, approximately 0.5 mL of phosphate-buffered saline (PBS) was added to a thawed specimen. Subsequently, the tissue pieces were minced with a scalpel into a suspension of small cell clumps and single cells. The clumps were discarded after sedimentation for 3 to 5 minutes in 5 mL of PBS. The supernatant was centrifuged, and the resulting pellet washed and resuspended in PBS. The cell suspension was incubated with hypotonic solution (0.075 mol/L potassium chloride) for 10 minutes at 37°C and fixed in methanol/acetic acid (3:1). Fixed cells were stored in methanol at −20°C until used for FISH. The previously described procedure was adequate for tissue processing, and fixation of nuclei from both primary tumor tissue and lymph node metastases. As nuclei from TUR tissue tended to coagulate in suspension, an additional sedimentation step at unit gravity was necessary for these preparations.

FCM Procedure

Samples were processed for DNA-FCM as described.[3] The ploidy of the different peaks in histograms from tumor samples was calculated from their position, relative to the G0/G1 peak (C = 2) in a histogram of cultured normal diploid prostate fibroblasts. Diploid: C = 1.9 to 2.2; hypodiploid C ≤ 1.8; hyperdiploid C = 2.3 to 2.7; triploid: C = 2.8 to 3.4; tetraploid: C = 3.5 to 4.2. Samples had a significant tetraploid cell population when the tetraploid peak, representing diploid G2/M as well as tetraploid tumor G0/G1 nuclei, contained more than 10% of the nuclei.

FISH Procedure

The chromosome-specific probes used were PUC 1.77 for chromosome 1[14]; p7t.1 for chromosome 7[15]; D8Z2 for chromosome 8[16]; D10Z1 for chromosome 10[17]; L1.84 for chromosome 18[18]; and DYZ5 (Amprobe; Amersham, Buckinghamshire, England) for the Y chromosome. Hybridization and detection were performed as described before.[2] Hybridization

TABLE 2. Combined Results of FISH Analysis of Six Centromere Probes in 11 BPH Specimens

	Loss of Signals*		Extra signals†		ANC		
						Cutoff§	
Chromosome	Mean % ± SD	Cutoff %‡	Mean % ± SD	Cutoff %‡	Mean ± SD	Gain	Loss
1	3.5 ± 2.4	8.3	2.1 ± 1.4	5.0	1.99 ± 0.03	2.08	1.90
7	2.4 ± 2.1	6.6	2.1 ± 1.0	5.0	2.00 ± 0.03	2.09	1.91
8	3.2 ± 2.5	8.2	1.8 ± 1.0	5.0	1.99 ± 0.03	2.08	1.90
10	2.6 ± 2.2	7.0	1.8 ± 1.3	5.0	2.00 ± 0.02	2.06	1.94
18	1.2 ± 1.1	5.0	2.2 ± 1.6	5.4	2.01 ± 0.02	2.07	1.95
Y	1.0 ± 1.3	5.0	4.4 ± 2.3	9.0	1.03 ± 0.03	1.12	0.94

Abbreviations: ANC, average number of copies; FISH, fluorescence in situ hybridization; BPH, benign prostatic hyperplasia; SD, standard deviation.

* Loss of signals: percentage of less than two spots for chromosomes 1, 7, 8, 10, and 18; less than one spot for Y.

† Extra signals: percentage of greater than two spots for chromosomes 1, 7, 8, 10, and 18; greater than one spot for Y.

‡ Cutoff percentage = mean + 2 × SD; at least 5%.

§ Cutoff ANC for gain = mean ANC + 3 × SD; cutoff ANC for loss = mean ANC − 3 × SD.

of the biotinylated probe (15 ng per slide) to the nuclei occurred during overnight incubation at 37°C in a moist chamber in 65% formamide for chromosomes 1, 18, and Y and in 60% formamide for chromosomes 7, 8, and 10.

Evaluation and Statistics

For the evaluation of FISH signals, the authors used the criteria defined by Hopman et al[19]: (1) nuclei should be intact and should not overlap; and (2) FISH signals within one nucleus should be completely separated (split or paired spots should be counted as one) and of the same intensity. When these criteria could not be met, such nuclei were excluded from counting. When more than 10% of the nuclei on a slide had to be excluded, the hybridization was repeated. When there were more than 5% nuclei with one spot on a slide, the hybridization was also repeated. At least 300 nuclei were scored per sample and per probe.

In mixed tumor-normal samples, small aberrations will not be detectable when the percentage of nontumor cells is too large. To compensate for this heterogeneity, only tumor samples that contained more than 50% tumor cells were electable for this study. So, depending on the cutoff percentage (range, 5% to 9%; Table 2), aberrations occurring in as few as 10% of the tumor cells could still be detected.

For each chromosome in each specimen, the average number of copies (ANC) was calculated (total number of spots counted/total number of nuclei counted). Consequently, an ANC of 0.90 for Y means that 10% of the nuclei in a tissue sample show no signal; an ANC of 2.15 means that a maximum of 15% of the nuclei show gain of one or more signals. The Kruskal-Wallis test was used to test the relation between the ANC and clinical stage.[11]

RESULTS

BPH Specimens

For the 11 BPH specimens investigated, the ANCs for each investigated chromosome were narrowly distributed around the diploid values (Table 2). The ranges found were 1.93 to 2.05 (chromosome 1), 1.94 to 2.03 (chromosome 7), 1.93 to 2.04 (chromosome 8), 1.96 to 2.05 (chromosome 10), 1.98 to 2.05 (chromosome 18), and 0.97 to 1.07 (Y chromosome).

Loss of signals as well as extra spots were generally scored with low frequency. Only the Y chromosome showed a relatively high mean (4.4%) for extra spots scored. Because no evidence for numerical abnormalities of the investigated chromosomes was shown, BPH could be considered a diploid control. Thus, the BPH results were used to assess cutoff ANCs (Table 2). Significant gain and loss (further referred to as gain and loss) of the investigated chromosomes in PC are defined as at or above cutoff ANC for gain and at or below cutoff ANC for loss, respectively.

PC Specimens

Detailed data per specimen and per chromosome are listed in Appendix 1. Significant numerical aberrations were detected for all six investigated chromosomes. Gain (trisomy and tetrasomy combined; occasionally, pentasomy and hexasomy) was more common than loss (monosomy and nullisomy). Gain was highest for chromosome 10 (64.7%) and lowest for chromosome Y (16.3%). Loss was highest for Y chromosome (14%) and lowest for chromosome 7 (2.6%). In metastatic tumors, more aberrations were detected than in nonmetastatic tumors. Five tumors did not show any abnormalities, whereas in 21 tumors one or more chromosomes showed simultaneous gain and loss. This resulted mostly in normal values for the ANC.

Chromosome 1

The results for chromosome 1 showed gain in 37.8% but loss in less than 10% of the tumors (Fig 1). Two cases (PC202 and PC302) showed simultaneous loss and gain. In metastatic tumors, gain was more frequent than in nonmetastatic tumors. Loss was found only in two metastatic tumors (PC295 and PC420). Polysomy was observed in all stage groups irrespective of long or short survival time of the patient (Table 1). Statistics did not show any correlation of numerical aberrations with increasing stage, metastatic disease, or tumor site (Table 3).

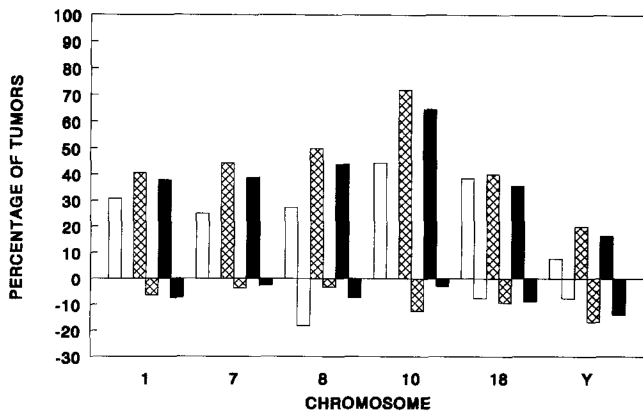

FIGURE 1. Percentages of gain and loss of chromosomes 1, 7, 8, 10, 18, and Y in nonmetastatic, metastatic, and all PC specimens, respectively. For chromosomes 1 and 18, all investigated tumor specimens could be evaluated (ie, 15 nonmetastatic and 31 metastatic specimens). For chromosomes 7, 8, 10, and Y, 14, 14, 11, and 15, respectively, nonmetastatic and 25, 29, 24, and 29, respectively, metastatic specimens were evaluable. Positive percentages (standing bars) represent gain; negative percentages (hanging bars) represent loss. □, Nonmetastatic; ▩, metastatic; ■ all.

Chromosome 7

Numerical aberrations of chromosome 7 were observed in 41% of the tumors. Although only one tumor (PC320) showed loss, gain was observed in 38.8% of the tumors. Seven tumors showed simultaneous loss and gain. Gain of chromosome 7 was more frequent in metastatic tumors, and a significant difference between the ANCs of nonmetastatic and metastatic tumors was found (Fig 1). However, high percentages of polysomy were observed in two patients that were disease free for more than 2 years (PC290 and PC342; Table 1). The ANCs of radical prostatectomy specimens and lymph node metastasis (LM) specimens were significantly different (Table 3).

Chromosome 8

The copy number of chromosome 8 was shown to be aberrant in 51% of the tumors. Gain of chromosome 8 was more frequently found in metastatic tumors, whereas loss occurred more often in nonmetastatic tumors (Fig 1). Seven of 10 tumors with loss also showed gain. Four of five LM showed high percentages of polysomy (PC244, PC269, PC270, and PC295; Table 1). All these patients died within 2 years. However, also in one specimen from a patient who was disease free for more than 2 years (PC342), a high percentage of polysomy was found. High percentages of monosomy were observed in two lymph node–positive T3 tumors (PC256 and PC352). Significant differences in ANC values were found between nonmetastatic and metastatic tumors (Fig 1), between radical prostatectomy and TUR specimens, and between radical prostatectomy and LM specimens (Table 3).

Chromosome 10

Gain of chromosome 10, the most frequently found numerical aberration (64.7% of the tumors) correlated with metastatic disease (Fig 1). Significant loss of chromosome 10 occurred in only one metastatic tumor (PC479). However, concurrent loss and gain of chromosome 10 was found in another 10 tumors. ANC values tended to increase with stage (P = .05) but showed no relation to the survival time. The ANCs of LM were significantly higher than those of radical prostatectomy specimens but were not different from TUR values (Table 3).

Chromosome 18

Gain and loss of chromosome 18 were about equally frequent in nonmetastatic and in metastatic tumors (Fig 1). High polysomy percentages were present in all stage groups, the highest values being found in patients with short survival (eg, PC269, PC270, and PC291; Table 1). Statistics did not reveal any correlation of numerical aberrations with tumor site (Table 3).

Y Chromosome

Aberrations of Y chromosome, equally divided between loss and gain, did not correlate with any clinical parameter (Table 3), although the frequency of loss was higher in metastatic tumors (Fig 1). Only two cases showed simultaneous loss and gain (PC290 and PC332). The mean age of PC patients with a tumor that showed loss of Y chromosome was 65.4 ± 13.2 (range, 54 to 86 years; N = 7). The mean age of patients without chromosome Y loss was 67.0 ± 10.2 (range, 49 to 93 years; N = 36).

DNA-FCM Compared With FISH Results of PC Patients With Follow-Up and Staging Data

Of the 24 specimens, investigated both by FISH analysis and DNA-FCM, seven tumors were DNA diploid, three were tetraploid, and 14 were aneuploid (Table 1). Six of seven DNA diploid tumors occurred in node-negative patients, whereas the three tetraploid tumors were all from node-positive patients. Aneuploid

TABLE 3. Correlations of ANC Values per Chromosome With Tumor Site

	P Values*		
Chromosome	RP/TUR†	RP/LM‡	TUR/LM§
1	.81	.41	.24
7	.09	**.04**	.29
8	**.03**	**.04**	.12
10	.08	**.03**	.26
18	.91	.22	.34
Y	.48	.54	.39

Abbreviations: ANC, average number of copies; RP, radical prostatectomy; TUR, transurethral resection; LM, lymph node metastasis.
* Kruskal-Wallis test; *P* values in bold typeface indicate statistical significance.
† RP vs TUR.
‡ RP vs LM.
§ TUR versus LM.

tumors were found in patients that were at least node positive or staged T3, except case no. 13 (T2N0). Investigated with FISH, five tumors did not show any numerical aberration. Two of these tumors were diploid (PC341 and PC382), and two were aneuploid (PC343 and PC371). The fifth (PC400) was not evaluable by FCM. All other tumors showed numerical aberrations of one or more chromosomes. The average number of aberrant chromosomes in diploid tumors was 2.0 ± 2.2, and in aneuploid and tetraploid tumors together was 4.7 ± 2.3. Chromosome gain was most prominent in highly DNA aneuploid tumors (PC244, PC269, PC270, PC290, PC291, PC295, PC342, and PC354). Tumors with hypodiploid cell populations could show chromosome loss (PC295), or not (PC269). However, some tumors that did not show hypodiploid cells with FCM (eg, PC202, PC256, and PC290) showed loss for more than one chromosome.

Cases With Multiple Specimens

PC256 and PC384 (case no. 4) showed a DNA histogram with about the same percentage of tetraploid cells (Fig 2). FISH showed numerical aberrations for all investigated chromosomes in both specimens, but loss of chromosome 7 and gain of chromosome 10 were more pronounced in the second sample PC384.

The two samples obtained from case no. 5 (PC269 and PC291) displayed a profound dissimilarity in FCM results, with different proportions of different DNA content (Fig 2). With FISH, all investigated chromosomes showed numerical aberrations in both samples. Although polysomy of chromosomes 1, 8, and especially 18 was far more extended in PC291, in PC269 chromosome 10 was more polysomic than in the second sample.

The two samples obtained from case no. 10 were also heterogeneous with respect to FCM results. In the TUR (PC320, sample contained 80% tumor cells), a triploid stemline (3.0C) was found, whereas in a sample from the xenografted cell line (PC295), which consequently consisted exclusively of tumor cells, a hypotetraploid (3.7C) and a hypodiploid stemline (1.4C) were observed (Fig 2). The histograms of the FISH results showed, however, that these tumors were clearly related: Both samples showed polysomy for chromosomes 8 and 10, and loss for more than one chromosome. Losses were more extensive in PC295 than in PC320, except for Y chromosome, which was completely lost in PC320 and only partly lost in PC295.

DISCUSSION

In the BPH samples tested, essentially no numerical aberrations were detected for the six chromosomes investigated. These results are in contrast with the report of Aly et al,[20] who found loss of chromosome Y and gain of chromosome 7 in several cultured BPH specimens. Based on the findings in the present study, the authors assumed that occasional BPH cells would not contribute to any chromosomal abnormalities found in PC specimens.

DNA-FCM studies on about one half of the tumors showed that the frequency of DNA aneuploidy increased with increasing tumor stage. Combined FISH and DNA-FCM showed that, as expected, most aneuploid tumors had numerical chromosomal aberrations. It was, however, also shown that most diploid tumors also had one or more numerically aberrant chromosomes. This can be explained by the fact that only chromosomal aberrations that constitute more than 4% of the total amount of DNA can be detected with DNA-FCM. No chromosome seemed to be specifically aberrant in DNA diploid tumors.

Forty-one of 46 PCs showed numerical aberrations of one or more chromosomes of the six chromosome panel. All investigated chromosomes individually showed numerical aberrations in at least 30% of the specimens, and gain was more frequent than loss. Gain of chromosome 10 was the most frequent numerical aberration found (65%); chromosome 8 was the second most frequently gained chromosome (44%); and chromosomes 1, 7, and 18 showed gain with a frequency between 35% and 40%. The Y chromosome showed the lowest frequency of gain (16%), but the highest frequency of loss (14%). Gain of chromosomes 7, 8, and 10 correlated with metastatic disease. Moreover, the ANC of chromosome 10 was significantly higher in advanced tumors. Gain of chromosome 10 in PC has been reported before,[10] and deletions of part of 10q have been reported in cytogenetic[1,21] and loss of heterozygosity (LOH) studies.[10,22-24] However, the association of chromosome 10 aberrations with metastatic disease is a new finding.

In a previous study, the authors of the present study were the first to suggest that 8p deletions are possibly important aberrations in PC.[25] Since then, 8p deletions have been reported in several cytogenetic studies of PCs.[1,10,26-28] Recently, the importance of loss of chromosome 8p sequences in PC and the putative presence of at least two tumor suppressor genes on chromosome 8p has been established by several LOH studies.[26-31] The deletions that were found by LOH mapping often spanned most of the 8p arm and sometimes involved the centromeric region. In the present study, loss of chromosome 8 was seen primarily in nonmetastatic tumors, whereas in metastatic tumors gain was more prominent.

Deletions of 7q are among the first aberrations reported in PC.[1] Gain of chromosome 7 has been found in cytogenetic[5,21,22,32] and FISH studies.[10,11,33,34] In agreement with the findings in the present study, Bandyk et al[11] observed that gain of chromosome 7 was significantly increased in PC metastases compared with primary tumors.

Loss of chromosomes 7, 8, and 10 was observed mostly simultaneously with gain in the same tumor samples, which is suggestive for heterogeneity in such tumors. In fact, chromosome 8 centromere gains and losses were shown to exist in different areas of the same tumors.[5] Loss of (part of) a homologue followed by multiplication of the other homologue is one possible mechanism through which these numerical aberrations could be generated. Another mechanism could be the formation of an isochromosome, followed by a nondisjunction event. This has been suggested as an explana-

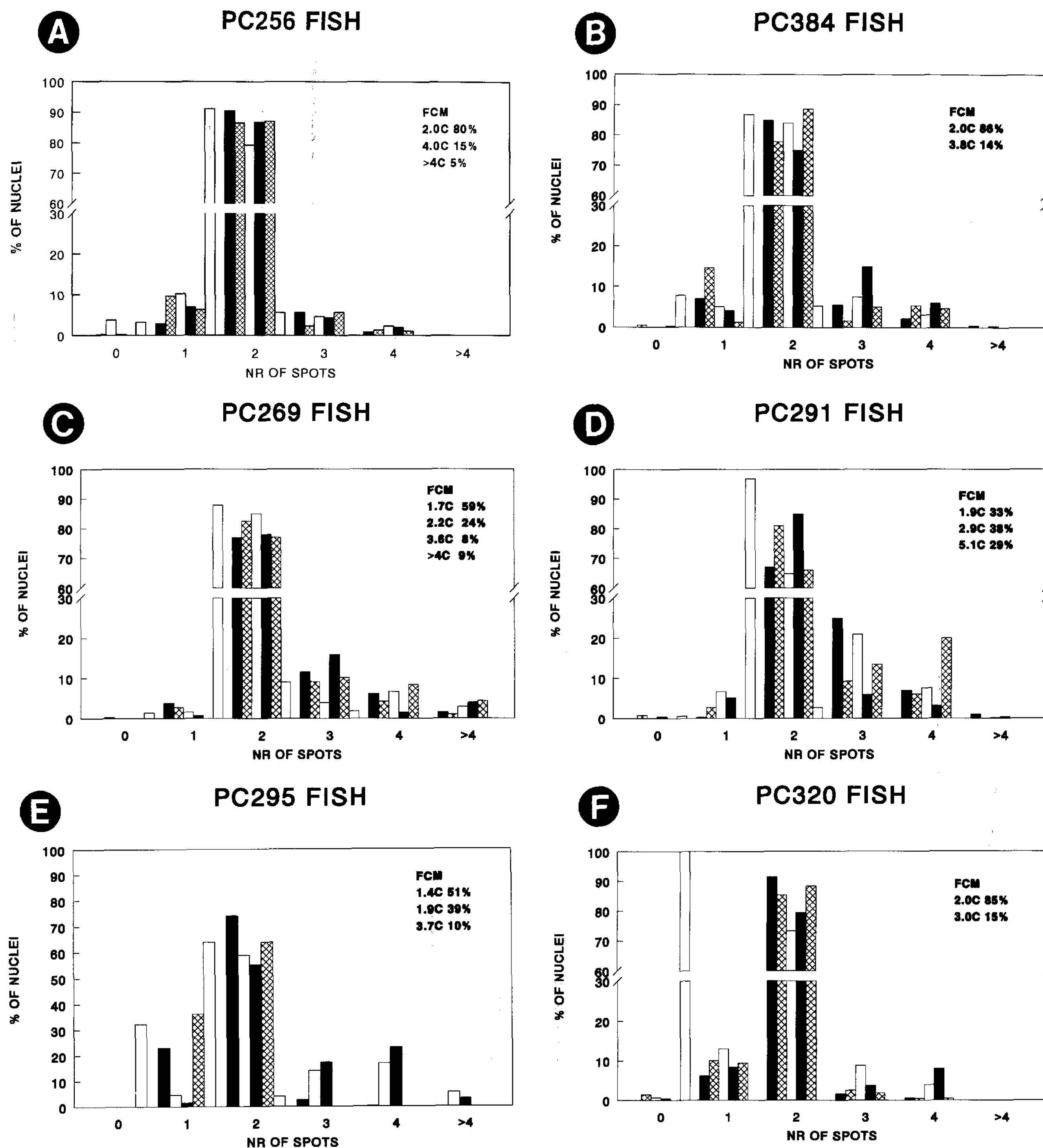

FIGURE 2. FISH and DNA-FCM results of cases with multiple specimens. (A and B) Case no. 4. (C and D) Case no. 5. (E and F) Case no. 10. ■ 1; ▨ 7; □ 8; ■ 10; ▨ 18; □ Y.

tion for the simultaneous loss of 8p and gain of 8q.[27] In a recent comparative genome hybridization study of PC, it was reported that loss of 8p and gain of 8q occur frequently, but that gain of (the whole) 7 and loss of 10q are infrequent observations.[35]

Chromosome 18 likewise showed simultaneous loss and gain in most tumors, suggesting similar possible mechanisms as stated previously. The percentage of numerical aberrations of chromosome 18, however, did not correlate with metastatic disease. Moreover, the authors have reported before that DNA diploid PCs, which mostly are early tumors, often already show gain of chromosome 18.[12] So numerical aberrations of chromosome 18 are likely to be an early event in PC. In fact, recently allelic losses of 18q were reported in six of 20 early-stage PCs.[36] It will be of interest to investigate if chromosome 18 aberrations are already present in the preneoplastic prostatic intraepithelial neoplasia lesions.

Aberrations of both chromosomes 1 and Y also did not correlate with metastatic disease. Furthermore, simultaneous loss and gain were only rarely observed. Previously, the authors[12] described that gain of chromosomes 1 and Y was largely restricted to DNA aneuploid tumors, and this finding was confirmed in the present study. Y loss in the tumor did not correlate with the patients age. So alternative mechanisms, like multiplication through tetraploidization of the whole genome[37] or loss of Y as a reflection of a general state of hyperproliferation,[38] are more likely explanations for the aberrations found for these chromosomes.

Comparison of two consecutive samples from the same patients revealed similar results with FCM in only one of three cases. However, FISH analysis of these specimens revealed clear evidence of karyotypic evolution toward a more aberrant karyotype.

The present article has substantiated the value of centromere FISH as a means to look at the ploidy of individual chromosomes in prostate cancer tissue. Numerical aberrations were found for all six investigated chromosomes; no doubt, most other chromosomes, when eventually investigated, will also show numerical aberrations in at least part of the PCs. The most important finding was, however, that combined with clinical data, gains of chromosomes 7, 8, and 10 were shown to be definitely involved in the progression of PC. This not only substantiates the postulated presence of tumor suppressor genes on 8p but also justifies renewed interest in the commonly deleted regions on 7q and 10q.[1]

APPENDIX 1. Average Number of Copies of Investigated Chromosomes per Tumor Sample

PC NR	ANC 1	ANC 7	ANC 8	ANC 10	ANC 18	ANC Y
Nonmetastatic tumors						
256†	2.05	1.95	1.92*	2.01*	2.02*	1.02
285	**2.08**	2.01	NE	NE	**2.11**	1.06
288	**2.11**	2.00	1.95*	**2.17**	1.99	**0.94**
290	**2.49**	**2.46**	**2.31***	**2.16***	**2.26**	1.02*
341‡	1.96	2.02	1.99	1.99	1.97	1.01
342	**2.32**	**2.38**	**2.51**	**2.12**	**2.17**	**1.22**
343‡	1.93	1.99	1.97	2.00	1.98	0.97
352	1.97	2.01	**1.90**	NE	1.96	1.01
354	2.03	**2.14**	**2.09**	**2.11**	**2.35**	1.02
362	**2.29**	2.02	**2.11**	2.05	2.03*	1.06
382	2.01	2.05	1.95	2.00	2.01	1.03
389	2.05	2.02*	2.00	**2.09***	**2.12**	1.08
395	1.95	1.93	2.03	1.97	**1.94**	**0.91**
432	1.96	1.98	**1.79**	NE	1.99	1.01
435‡	2.04	NE	2.02	NE	2.04	1.09
Metastatic Tumors						
202	2.03*	2.07*	1.98*	1.95*	**2.08**	0.97
236	2.04	2.06	2.02	**2.12**	1.99	1.01
244	2.03	**2.37**	**2.31**	**2.34**	**2.35**	0.98
262	2.06	2.08	NE	2.02*	1.97	NE
269†	**2.24**	**2.17**	**2.24**	**2.30**	**2.40**	1.11
270	**2.30**	**2.28**	**2.80**	**2.44**	**2.36**	**1.26**
289	**2.28**	**2.12**	1.91	2.03	**2.29**	1.06
291†	**2.42**	**2.17**	**2.30**	**2.08***	**2.53**	1.02
295†	**1.80**	NE	**2.60**	**2.71**	**1.64**	**0.72**
296	2.01	2.06	**2.11**	NE	2.02*	1.05
302	2.06*	2.03*	**2.11**	**2.21***	**2.17**	0.99
320†	1.97	**1.90**	2.04*	**2.11***	**1.93**	**0**
324	2.04	2.08	2.03	**2.11**	**1.94**	**0.21**
332	2.07	NE	1.99*	NE	1.98	1.05*
351	**2.20**	**2.52**	**2.34**	**2.51**	**2.12**	1.11
353	**2.09**	**2.28**	NE	**2.36**	1.99*	NE
355	**2.37**	**2.09***	**2.12***	**2.10**	**2.29**	1.03
366	**2.28**	**2.35**	**2.13**	**2.23**	**2.14***	**0.79**
371‡	2.00	2.04	1.99	2.03	2.00	0.98
384†	**2.16**	1.97*	**2.13**	**2.23**	**2.12**	0.97
392	2.00	2.07	2.00*	NE	2.01	1.01
400‡	1.94	2.06	1.95	NE	2.01	1.02
403	1.96	2.01*	2.07	2.03*	1.98	1.08
405	2.04	NE	**2.09**	NE	1.98	**1.15**
417	1.95	NE	**2.17**	NE	2.05	1.02
418	2.03	**2.48**	**2.27**	**2.21**	**2.49**	1.03
420	**1.90**	NE	2.06	**2.14***	1.98	1.00
449	**2.18**	**2.21**	**2.09**	NE	2.03*	**1.27**
461	2.01	NE	2.03	**2.06**	2.01	**0.92**
465	**2.26**	**2.18**	**2.33**	**2.14**	**2.18***	**1.23**
479	1.96	1.98*	**1.89**	**1.85**	**1.90**	1.00

Abbreviations: NE, not evaluable; PC, prostate carcinoma; NR, number; ANC, average number of copies; bold figures, significant gain or loss.

* Simultaneous significant gain and loss.

† Specimen from double-sampled patient.

‡ Specimen without chromosomal aberrations.

REFERENCES

1. Sandberg AA: Chromosomal abnormalities and related events in prostate cancer. HUM PATHOL 23:368-380, 1992
2. König JJ, Teubel W, van Dongen JJW, et al: Tissue culture loss of aneuploid cells from carcinomas of the prostate. Genes Chrom Cancer 8:22-27, 1993
3. König JJ, van Dongen JJW, Schröder FH: Preferential loss of abnormal prostate carcinoma cells by collagenase treatment. Cytometry 14:805-810, 1993
4. Breitkreuz T, Romanakis K, Lutz S, et al: Genotypic characterization of prostatic carcinomas: A combined cytogenetic, flow cytometry, and in situ DNA hybridization study. Cancer Res 53:4035-4040, 1993
5. Micale MA, Sanford JS, Powell IJ, et al: Defining the extent and nature of cytogenetic events in prostatic adenocarcinoma: Paraffin FISH vs metaphase analysis. Cancer Genet Cytogenet 69:7-12, 1993
6. Brothman AR, Watson MJ, Zhu XL, et al: Evaluation of 20 archival prostate tumor specimens by fluorescence in situ hybridization (FISH). Cancer Genet Cytogenet 75:40-44, 1994
7. Baretton GB, Valina C, Vogt T, et al: Interphase cytogenetics of prostatic carcinomas by use of nonisotopic in situ hybridization. Cancer Res 54:4472-4480, 1994
8. Zincke H, Bergstralh EJ, Larson-Keller JJ, 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 70:312-323, 1992
9. Lundgren R, Heim S, Mandahl N, et al: Chromosome abnormalities are associated with unfavorable outcome in prostate cancer patients. J Urol 147:784-788, 1992
10. Macoska JA, Micale MA, Sakr WA, et al: Extensive genetic alterations in prostate cancer revealed by dual PCR and FISH analysis. Genes Chromos Cancer 8:88-97, 1993
11. Bandyk MG, Zhao L, Troncoso P, et al: Trisomy 7: A potential cytogenetic marker of human prostate cancer progression. Genes Chromos Cancer 9:19-27, 1994
12. König JJ, Teubel W, van Dongen JJW, et al: Loss and gain of chromosomes 1, 18 and Y in prostate cancer. Prostate 25:281-291, 1994
13. Schröder FH, Hermanek P, Denis L, et al: The TNM classification of prostate cancer. Prostate 4:129-138, 1992 (suppl)
14. Cooke HJ, Hindley J: Cloning of human satellite III DNA: Different components are on different chromosomes. Nucl Acids Res 6:3177-197, 1979
15. Waye S, England SB, Willard HF: Genomic organization of α satellite DNA on human chromosome 7: Evidence for two distinct alphoid domains on a single chromosome. Mol Cell Biol 7:349-356, 1987
16. Donlon T, Wyman AR, Mulholland J, et al: α Satellite-like sequences at the centromeres of chromosome #8. Am J Hum Genet 39:A196, 1986
17. Devilee P, Kievits T, Waye JS, et al: Chromosome-specific α satellite DNA: Isolation and mapping of a polymorphic alphoid repeat from human chromosome 10. Genomics 3:1-7, 1988
18. Devilee P, Cremer T, Slagboom P, et al: Two subsets of human alphoid repetitive DNA show distinct preferential localization in the pericentric regions of chromosomes 13, 18 and 21. Cytogenet Cell Genet 41:193-201, 1986
19. Hopman AHN, Ramaekers FCS, Raap AK, et al: In situ hybridization as a tool to study numerical chromosome aberrations in solid tumors. Histochemistry 89:307-316, 1988
20. Aly M, Dal Cin P, van de Voorde W, et al: Chromosome abnormalities in benign prostatic hyperplasia. Genes Chromos Cancer 9:227-233, 1994
21. Arps S, Rodewald A, Schmalenberger B, et al: Cytogenetic survey of 32 cancers of the prostate. Cancer Genet Cytogenet 66:93-99, 1993
22. Bergerheim USR, Kunimi K, Collins VP, et al: Deletion mapping of chromosomes 8, 10, and 16 in human prostatic carcinoma. Genes Chrom Cancer 3:215-220, 1991
23. Carter BS, Ewing CM, Ward WS, et al: Allelic loss of chromosomes 16q and 10q in human prostate cancer. Proc Natl Acad Sci USA 87:8751-8755, 1990
24. Kunimi K, Bergerheim USR, Larsson IL, et al: Allelotyping of human prostatic adenocarcinoma. Genomics 11:530-536, 1991
25. König JJ, Kamst E, Hagemeijer A, et al: Cytogenetic characterization of several androgen responsive and unresponsive sublines of the human prostatic carcinoma cell line LNCaP. Urol Res 17:79-86, 1989
26. Macoska JA, Trybus TM, Sakr WA, et al: Fluorescence in situ hybridization analysis of 8p allelic loss and chromosome 8 instability in human prostate cancer. Cancer Res 54:3824-3830, 1994
27. Cher ML, MacGrogan D, Bookstein R, et al: Comparative genomic hybridization, allelic imbalance, and fluorescence in situ hybridization on chromosome 8 in prostate cancer. Genes Chromos Cancer 11:153-162, 1994
28. Matsuyama H, Pan Y, Skoog L, et al: Deletion mapping of chromosome 8p in prostate cancer by fluorescence in situ hybridization. Oncogene 9:3071-3076, 1994
29. Bova GS, Carter BS, Bussemakers MJG, et al: Homozygous deletion and frequent allelic loss of chromosome 8p22 loci in human prostate cancer. Cancer Res 53:3869-3873, 1993
30. MacGrogan D, Levy A, Bostwick D, et al: Loss of chromosome arm 8p loci in prostate cancer: Mapping by quantitative allelic imbalance. Genes Chromos Cancer 10:151-159, 1994
31. Trapman J, Sleddens HFBM, van der Weiden MM, et al: Loss of heterozygosity of chromosome 8 microsatellite loci implicates a candidate tumor suppressor gene between the loci D8S87 and D8S133 in human prostate cancer. Cancer Res 54:6061-6064, 1994
32. Micale MA, Mohamed A, Sakr W, et al: Cytogenetics of primary prostatic adenocarcinoma: Clonality and chromosome instability. Cancer Genet Cytogenet 61:165-173, 1992
33. Jones E, Zhu XL, Rohr LR, et al: Aneusomy of chromosome 7 and 17 detected by FISH in prostate cancer and the effects of selection in vitro. Genes Chromos Cancer 11:163-170, 1994
34. Alcaraz A, Takahashi S, Brown JA, et al: Aneuploidy and aneusomy of chromosome 7 detected by fluorescence in situ hybridization are markers of poor prognosis in prostate cancer. Cancer Res 54:3998-4002, 1994
35. Visakorpi T, Kallioniemi A, Syvanen A, et al: Genetic changes in primary and recurrent prostate cancer by comparative genomic hybridization. Cancer Res 55:342-347, 1995
36. Latil A, Baron J, Cussenot O, et al: Genetic alterations in localized prostate cancer: Identification of a common region of deletion on chromosome arm 18q. Genes Chromos Cancer 11:119-125, 1994
37. Shackney SE, Smith CA, Miller BW, et al: Model for the genetic evolution of human solid tumors. Cancer Res 49:3344-3354, 1989
38. Casalone R, Portentoso P, Granata P, et al: Chromosome changes in benign prostatic hyperplasia and their significance in the origin of prostatic carcinoma. Cancer Genet Cytogenet 68:126-130, 1993