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Loss and Gain of Chromosomes 1, 18, and Y in Prostate Cancer

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ABSTRACT: Nuclear suspensions of 42 prostate carcinoma specimens obtained at surgery were used to investigate loss and gain of chromosomes 1, 18, and Y by fluorescence in situ hybridization (FISH) with centromere-specific probes. The outcome of FISH analysis was correlated with clinical parameters and the relationship between DNA-FCM (ploidy at cellular level) and FISH (ploidy of individual chromosomes) was assessed.

Significant loss of chromosomes 1 and 18 was infrequent (respectively, three and five cases), but 53% of the tested specimens showed loss of Y. Loss was not correlated with DNA ploidy. Significant gain occurred in 36% (chromosome 1), 63% (chromosome 18), and 28% (Y) of the specimens. Gain of chromosome 18 was shown in DNA diploid (7/14) and aneuploid tumors (18/26), while gain of chromosomes 1 and Y was nearly restricted to DNA aneuploid specimens. Significant unbalance between these chromosomes occurred in 11 cases. Most cases which had significant gain of chromosome 1 or 18 showed trisomic as well as tetrasomic cells. Simultaneous loss of some and gain of other investigated chromosomes is suggestive of clonal heterogeneity and/or multiclonality. This was observed in eight tumors.

Correlation between DNA-FCM and FISH was best for the Y chromosome. DNA-FCM showed more aberrant histograms with increasing stage and grade of tumors. The presence of numerical aberrations of the investigated chromosomes however, seemed independent of clinical grade or stage. © 1994 Wiley-Liss, Inc.

KEY WORDS: **fluorescence in situ hybridization, molecular cytogenetics, interphase cytogenetics**

INTRODUCTION

In prostate cancer (PC), investigation of chromosomal aberrations might provide clues to the changes that play an important role in the origin and progression of this tumor. Classical cytogenetic studies, which mostly made use of short-term tissue culture to obtain an adequate number of metaphase spreads, have shown chromosomal aberrations in only a minority of cases [1–5]. Recurrent structural changes were reported for chromosomes 1, 2, and 7 (p and q arm), 3p, 6p, 8p, 10q, 13q, 15q, and 16q. Whole chromosome gain of nos. 7, 14, 20, and 22 and loss of one homolog of nos. 1, 2, 4, 5, and Y were most common [6]. However, most cases showed normal diploid karyotypes, probably due to selective isolation and preferential growth of normal epithelial cells [7, 8].

An alternative method to study chromosomal abnormalities is by measurement of the total DNA content of individual cells using DNA flow cytometry (FCM). Unlike cytogenetic analyses, such studies showed that most prostate tumors had one or more aneuploid cell populations [9–12], which was associated with an unfavorable outcome of the disease. The cytogenetic sensitivity of DNA-FCM is limited however: numerical or structural changes of individual chromosomes remain undetected.

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In the present study, we used a third technique for the investigation of chromosomal changes, namely fluorescence in situ hybridization (FISH) with chromosome-specific DNA probes [13]. FISH has the advantage over classical cytogenetics that tissue culture is not needed, because signals can be detected in interphase nuclei as well as in metaphases. Furthermore, the larger number of cells that can be analyzed, allows the discrimination between random abnormalities (which are frequently observed in karyotyping studies of PC [1, 2, 4]) and clonal changes. We have shown before that FISH on fresh nuclear suspensions of PC specimens is feasible [8]. FISH studies with centromere-specific probes investigate the copy number of particular chromosomes, to establish whether the chromosome copy number is simply a reflection of the total ploidy [14] or an indication for gene loss, regardless of whether a chromosome is lost (loss of one homolog) or gained (chromosomal duplication driven by gene loss on one homolog).

We report here the frequencies of loss and gain of chromosomes 1, 18, and Y. The rationale for studying these chromosomes was: 1) for chromosome 1 not only structural abnormalities but also whole chromosome loss and gain have been reported in PC [6], 2) in two separate studies loss of heterozygosity (LOH) was reported on the long arm of chromosome 18 [15, 16], 3) loss of Y was frequently found in PC [1–3, 5, 17], sometimes even as the only aberration. The results of these studies were correlated with tumor grade and stage, and DNA-FCM.

MATERIALS AND METHODS

Tissue Processing

Forty-two prostatic adenocarcinoma specimens were studied, obtained at radical prostatectomy (N = 25), transurethral resection (TUR; N = 13), or pelvic lymph node dissection (N = 4). Suspected carcinoma tissue was excised and, whenever the amount of tissue allowed, cut with scissors into two parts. One part was cut into several smaller fragments, snap frozen in liquid nitrogen, and stored at −80°C for later use. To the other part approximately 0.5 ml of phosphate buffered saline (PBS) was added and the tissue pieces were further minced with a scalpel into a suspension of small cell clumps and single cells. The clumps were discarded after sedimentation for 3–5 min in 5 ml of PBS. The supernatant was centrifuged and the resulting pellet washed and resuspended in PBS. From this cell suspension a sample was stored for DNA-FCM. The rest of the cell suspension was incubated with hypotonic solution (0.075 M KCl) for 10 min 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 above described procedure for tissue processing and fixation of nuclei gave good results with both primary tumor tissue and lymph node metastases (LM). As nuclei from TUR tissue tended to coagulate in suspension, an additional sedimentation step at unit gravity was performed with these preparations.

Histology was taken from adjacent tissue, in between the immediately processed sample and the sample for storage. From a paraffin section of each tumor, grade, stage, and percentage of tumor cells were assessed. The mean percentage of tumor cells in the sections was 56.3 ± 22.1% (range 25–95%). Specimens with less than 25% tumor cells were not included in the study.

For control purposes, leukocytes were obtained from each patient. A sample of peripheral blood in heparin was mixed with a 3% solution of high molecular weight dextran in saline (one part on two to three parts of blood). The erythrocytes were allowed to sediment at unit gravity for 15–30 min and the leukocytes in the supernatant were washed once with RPMI medium by centrifugation. The enriched leukocytes were then suspended in fetal calf serum with 12% dimethylsulfoxide and stored in liquid nitrogen.

DNA-FCM Procedure

Stored samples were processed for DNA-FCM as described [7]. The ploidy of the different peaks in histograms from tumor samples was calculated from their position, relative to the G0/G1 peak (C-value = 2) in a histogram of cultured normal diploid prostate fibroblasts. Diploid: C = 1.9–2.2; hypodiploid: C ≤ 1.8; hyperdiploid: C = 2.3–2.7; triploid: C = 2.8–3.4; tetraploid: C = 3.5–4.2. Tumors showing more than 10% of the nuclei to be in the tetraploid range, representing diploid G2M phase cells as well as tetraplolid tumor cells, were considered to comprise a significant population of tetraploid tumor cells [7].

FISH Procedure

The chromosome-specific probes used were: PUC 1.77 [18], hybridizing to SATIII repeats on the pericentromeric heterochromatin region of chromosome 1; L1.84 [19], hybridizing to alphoid repeats on the centromere of chromosome 18; and DYZ3 [20], hybridizing to SATIII repeats on the centromere of the Y chromosome.

Hybridization and detection were performed as described by Pinkel et al. [21] with modifications. In short, the probe was labeled with Bio-11-dUTP by nick translation. Slides with fixed nuclei were pretreated with RNase and postfixed with formalde-

TABLE I. Values of BPH Specimens Obtained by Fish Analysis*

Chromosome no.	No.	Loss[a]	Cut-off (% loss)[b]	Normal copy no.	Gain[a]	Cut-off (% gain)[b]
1	15	3.3 ± 2.5	8.3	93.7 ± 3.5	2.8 ± 3.3	9.4
18	12	2.4 ± 2.3	7.0	96.0 ± 2.6	1.7 ± 1.0	3.7
Y	11	0.9 ± 1.2	3.3	94.2 ± 1.9	2.6 ± 2.8	8.2

*At least 300 nuclei were scored per chromosome and per sample.
[a]All numbers are average percentages ± S.D. Loss: % 0+1 spot for chromosomes 1 and 18, 0 spots for Y; normal copy number: 2 spots for chromosomes 1 and 18, 1 spot for Y; gain: >2 spots for chromosomes 1 and 18, >1 spot for Y.
[b]Average + 2 × S.D.

hyde. Hybridization of the probe to the nuclei (15 ng probe per slide) occurred during overnight incubation at 37°C in a moist chamber in 65% formamide. Slides were subsequently washed in 60% formamide/2 × SSC (3 × 5 min), 2 × SSC (3 × 5 min) both at 40°C, followed by 3 × 5 min in 0.1 × SSC at 60°C, 5 min in 2 × SSC, and 5 min in 4 × SSC/0.05% Tween at 20°C. Detection of hybridized sequences was with Avidin-FITC (incubation: 20 min at 37°C), followed by biotinylated goat α-Avidin (incubation: 30 min at 37°C) and a second Avidin-FITC incubation. Nuclei were counterstained with propidium iodide in antifade solution. The number of spots per nucleus, representing the number of centromeric regions of a respective chromosome, was counted at ×1,250 magnification on a Zeiss Axiophot fluorescence microscope equipped with a FITC filter combination.

Evaluation and Statistics

Due to the limited amount of tissue available, hybridization with the probe for Y was not possible with 10/42 specimens. In two specimens the hybridization with the probe for chromosome 18 was not evaluable.

For the evaluation of FISH signals we used the criteria of Hopman et al. [22]: a) nuclei should be intact and should not overlap; b) 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.

Aneusomy was defined to be significant when the percentage of nuclei with numerical changes differed from the average percentage in benign prostatic hyperplasia (BPH) specimens by at least 2 times S.D. (Table I). Dependent on the number of nuclei counted, the Kolmogorov-Smirnov (KS) test [23] gives a minimally required discrepancy percentage for two observations. When two observations (in this study counts of spots from probes of two different chromosomes) differed more than the discrepancy percentage, these observations were considered statistically significant.

RESULTS

Frequency of Chromosomal Aberrations in BPH Specimens

The frequency of numerical aberrations of chromosomes 1, 18, and Y was determined in BPH specimens in order to gain insight in: 1) the sensitivity of the hybridization reaction for each probe, and 2) to assess cut-off percentages for significant gain and loss of each investigated chromosome.

Sensitivity was considered good for chromosomes 1 and 18 and very high for chromosome Y (see Table I, column headed "loss"). Extra spots (see Table I, column headed "gain") were scored with an acceptable frequency for chromosome 18 (range 0.3–3.5%), but the frequencies for chromosomes 1 (range 0.8–12.7%) and Y (range 2.5–7.2%) were higher.

The distribution of hybridization spots in nuclei from BPH specimens was used to calculate the cut-off values for significant gain or loss of each of the three chromosomes investigated in PC specimens (see Table I).

Frequency of Chromosomal Loss and Gain in PC Specimens

Figure 1A and Table II show the percentages of nuclei with less than two copies (for chromosomes 1 and 18, respectively) and less than one copy (Y chromosome), scored for each tumor individually. The average percentages were 3.1 ± 2.9 (1), 3.6 ± 3.2 (18), and 7.9 ± 17.6 (Y). Note that in one tumor the Y chromosome was entirely lost (PC320, see also Table

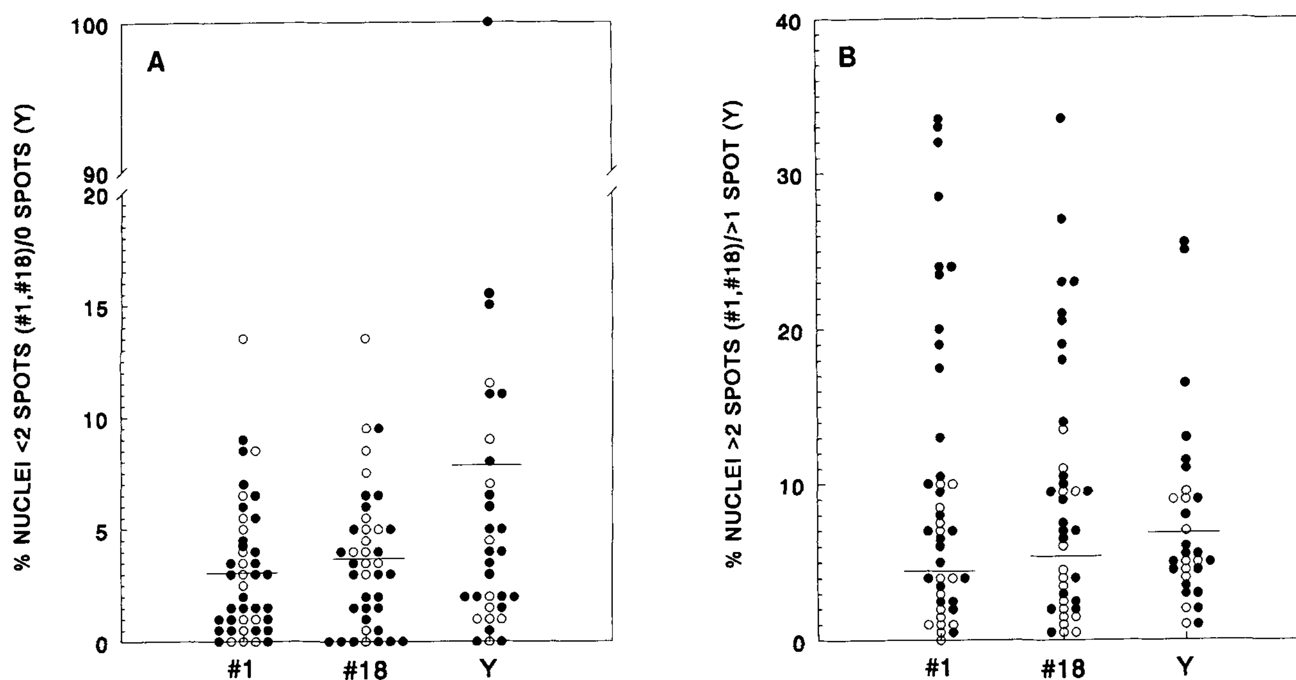

Fig. 1. **A:** Distribution of percentages of mono-nullisomic nuclei, and **B:** polysomic nuclei for chromosomes 1, 18, and Y. Open symbols, DNA diploid; closed symbols, DNA aneuploid; solid line, average percentage.

II). Since no loss of Y was observed in cultured peripheral lymphocytes of this patient, this loss was considered tumor specific.

Chromosome loss above cut-off level was infrequent for chromosomes 1 and 18, as it occurred in only 3/42 tumors for chromosome 1 and in 5/40 tumors for chromosome 18 (see Table II). Significant loss of the Y chromosome however, occurred in 17/32 tested specimens (53%; see Table II). Unbalanced loss, as determined by the KS test, occurred in four samples. In each of these cases, the Y chromosome was involved. In one case (PC320), also chromosome 18 was underrepresented. Hypodiploid populations (by DNA-FCM) were not detected in either of these tumors.

Figure 1B and Table III show the frequencies of polysomy of chromosomes 1, 18, and Y in the investigated tumors. Table III also shows the percentages and C-values of the aneuploid cell populations determined by DNA-FCM. The average percentages were, respectively, 4.0 ± 3.6, 4.6 ± 4.2, and 5.6 ± 3.0 for chromosomes 1, 18, and Y. Using the BPH values as a reference (see above), we observed significant gain in 16/42 (38%) cases for chromosome 1 and in 25/40 (63%) cases for chromosome 18. Significant gain of Y was less common: 9/32 tumors (28%). Two diploid tumors each showed gain of chromosomes 1 (PC288 and PC323) and Y (PC323 and PC360), while gain of chromosome 18 occurred in 7/14 diploid tumors. In 7/32 tumors gain was homogeneous, i.e., all three chromosomes showed significant polysomic populations (PC269, PC270, PC289, PC290, PC323, PC342, and PC351). Significant unbalance among chromosomes 1, 18, and Y, as determined by the KS test, occurred in nine tumors (PC288, PC290, PC291, PC292, PC301, PC342, PC344, PC354, and PC362). Populations of penta- and/or hexasomic nuclei of ≥1% were found in PC269, PC289, PC290, and PC291 for chromosome 1, PC269 and PC354 for chromosome 18, and PC269 and PC290 for the Y chromosome (see also Fig. 2D). In PC301 and PC353 (both DNA diploid), loss as well as gain of chromosome 18 was found. Simultaneous loss and gain of chromosome 1 was found in PC202, whereas simultaneous loss and gain of chromosome Y was found in PC289, PC290, PC296, PC342, and PC360.

From four patients two tissue samples were obtained. From one of these the samples were obtained from the primary tumor (PC256) and from a local recurrence, 34 months after radical prostatectomy (PC384). FCM and FISH results of these samples were comparable; in both samples an equal percentage of aneuploid cells was present and chromosome 18 showed a population of polysomic cells. From three other patients a lymph node metastasis (LM) and a sample from the primary tumor at palliative surgery

TABLE II. Loss of Chromosomes 1, 18, and Y Using FISH*

PC no.	% of nulli-monosomic nuclei for target chromosome		
	1	18	Y
202	8.3	4.8	11.2
288[a]	0.0	4.2	11.3
289	0.6	0.0	6.4
290[a]	0.4	0.6	15.5
295	0.7	14.2	ND
296	3.4	6.4	4.9
301	13.3	9.4	ND
303	1.3	NE	11.2
308	0.8	0.7	3.4
320[a]	6.2	9.5	100
329	2.4	4.4	9.2
342	2.0	2.9	3.7
343	9.0	3.8	4.1
353	2.7	8.6	ND
359[a]	5.4	NE	15.2
360	3.5	3.2	4.6
371	6.5	2.8	5.7
384	7.0	1.4	7.2
395	6.3	7.3	7.2
396	3.0	1.6	4.9

*Underlined percentages are above cut-off values. ND, not determined; NE, not evaluable. For DNA-FCM results of these samples, see Table III.
[a]Unbalanced chromosome loss.

(TUR) were available. One of these patients showed a high percentage of polysomy for chromosome 18 in the TUR material (PC301), but not in the LM (PC236), while FCM results were comparable in both samples. Another patient showed no difference with FISH, while FCM was different (PC295 and PC320). The third patient showed complex, but different FCM in both samples and a profound dissimilarity with FISH (PC269 and PC291).

Correlation Between DNA-FCM and FISH Results

In 10/14 tumors which showed a diploid DNA-FCM histogram, significant chromosomal aberrations were found (Tables II, III). One specimen (PC323) showed polysomy for all three chromosomes. Significant chromosomal aberrations were detected in 26/28 tumors that were aneuploid by DNA-FCM. In most aneuploid tumors the percentage of aneuploidy, determined by DNA-FCM, was higher than the percentage of aneusomic nuclei. This was true for all three chromosomes. Only in two cases (PC362 and PC292) the percentage of polysomy found for chromosome 1 was two times higher than the percentage of aneuploid cells, as determined by FCM. This was not found for either chromosome 18 or Y.

When polysomy of chromosomes 1, 18, and/or Y was detected, in nearly all cases trisomic as well as tetrasomic nuclei for chromosomes 1 and 18, and di- and trisomic nuclei for the Y chromosome were found, irrespective of whether the total DNA content was DNA-tetraploid (PC236–384 in Table III), DNA-triploid (PC202–371), or otherwise aneuploid (PC269–396). Figure 2 shows the spot distributions of chromosomes 1, 18, and Y for representative tumors with different ploidy patterns. Six DNA histograms of PC specimens showed populations of hypodiploid cells (PC269, PC292, PC295, PC306, PC308, and PC396; see Table III). PC295, PC308 and PC396 showed loss of a chromosome (see Table II).

Figure 3 shows the relationship between the percentage of aneuploid nuclei found with DNA-FCM and the percentage of polysomic nuclei found with FISH. The correlation between DNA-FCM and FISH of chromosomes 1 and 18 was about equal (respectively, 0.531 and 0.529), but the correlation between DNA-FCM and polysomy for the Y chromosome was better ($r = 0.78$). All correlations were highly significant ($P < 0.0005$).

Relationship of Stage and Grade With Numerical Aberrations and Ploidy

Aberrations of chromosomes 1, 18, and/or Y were equally distributed over all stages and grades, while with increasing grade or stage FCM showed more aberrant histograms (see Tables IV, V).

DISCUSSION

We investigated the frequencies of loss and gain of chromosomes 1, 18 and Y by FISH and the total ploidy by DNA-FCM in the same nuclear suspension, obtained from tissue of patients with clinically evident PC. These three chromosomes are each, in one way or another, involved in the genetics of PC (see below).

Significant loss of chromosomes 1 and 18 was infrequent, and was not correlated with detectable loss of total DNA. Due to the high sensitivity of hybridization of Y in BPH specimens, the cut-off value was very low, so 17/32 PC specimens showed significant loss of Y. However, six of these tumors showed a loss percentage below 5%. The biological significance of such small subpopulations (≤5%) is presently unknown; clonal progression is a possibility, but technical artefacts cannot be completely excluded.

Significant gain of chromosomes 1 and Y was largely restricted to DNA aneuploid tumors. Around one third of the DNA aneuploid tumors showed gain

TABLE III. Gain of Chromosomes 1, 18, and Y and FCM Results*

	% of polysomic nuclei[a]			FCM			
				Major aneuploid population[b]		Other aneuploid population[c]	
PC no.	1	18	Y	%	C	%	C
285	7.0	11.0	6.9	7	3.9	0	—
288[d]	10.0	2.3	4.8	6	3.5	0	—
301[d,e]	2.8	9.3	ND	8	3.8	0	—
323	10.0	9.3	9.5	7	3.5	0	—
329	3.9	4.4	5.1	9	3.0	0	—
340	1.0	1.4	ND	7	3.1	0	—
341	0.1	0.4	1.0	2	3.4	0	—
352	1.1	2.0	1.8	6	2.9	0	—
353	7.3	5.7	ND	6	3.3	0	—
357	4.2	0.4	ND	7	3.0	0	—
360	0.3	3.4	8.7	3	3.6	0	—
382	1.5	4.0	4.2	0	—	0	—
389	8.3	13.5	0.0	9	3.9	0	—
395	2.0	0.9	4.4	4	3.8	0	—
236[e]	4.1	2.5	3.0	11	4.0	0	—
256[e]	6.6	6.8	5.6	15	4.0	0	—
270	23.6	27.0	25.6	70	3.8	10	4.8
286	10.0	14.0	ND	11	3.6	0	—
289	19.9	18.7	11.3	8	3.6	3	4.4
303	33.6	NE	5.1	10	3.8	0	—
355	24.2	21.2	4.6	37	3.6	0	—
362[d]	28.4	6.6	5.3	9	3.6	5	>4
384[e]	7.8	9.9	5.3	14	3.8	0	—
202	9.6	9.5	8.1	25	3.2	0	—
290[d]	33.0	20.4	16.3	45	3.2	15	3.6
						18	5.3
291[d,e]	32.0	33.3	2.8	38	3.1	29	5.4
296	3.4	6.8	9.1	8	2.9	6	3.8
318	5.8	3.2	ND	17	3.3	9	>4.2
320[e]	2.3	2.3	0.0	15	3.0	0	—
342[d]	23.8	7.4	24.7	81	3.0	10	4.7
343	1.8	2.0	0.7	22	2.9	0	—
351	12.9	10.6	13.0	34	3.1	0	—
354[d]	2.3	22.9	4.4	46	2.9	7	4.4
359	10.3	NE	5.7	29	3.4	3	>4
371	4.9	1.7	3.5	12	3.4	4	4.0
269[e]	19.1	23.0	10.8	59	2.2	24	1.7
						8	3.6
						9	5.8
292[d]	17.6	8.7	ND	69	1.8	9	3.5
295[e]	0.9	1.4	ND	38	1.5	10	3.9
						3	3.2
306	6.8	9.3	ND	77	1.8	3	3.5
308	7.2	3.7	4.7	66	1.7	4	3.1
344[d]	4.0	17.9	ND	54	2.6	9	3.4
396	0.4	0.4	1.8	94	1.6–2.0	6	3.6

*PC numbers are listed chronologically, but are grouped according to the main aneuploid population detected by FCM. ND, not determined; NE, not evaluable. Underlined percentages are above cut-off values.

[a]For chromosomes 1 and 18: percentage of nuclei >2 spots; for Y: percentage of nuclei >1 spot.

[b,c]Aneuploid ≠ diploid (1.9–2.2); (=2=G0/G1 peak position of normal cells) major population: with highest percentage.

[d]Unbalanced chromosome gain.

[e]PC236 and PC301, PC256 and PC384, and PC295 and PC320 are tissue samples from the same patients, sequentially obtained (see text).

of chromosome 1 or Y. Gain of chromosome 18 occurred in more than half of the cases, but the cut-off percentage set through the results obtained with BPH specimens was lowest for chromosome 18. Furthermore, the presence of trisomic cells together with tetrasomy for chromosomes 1 and 18 was a common finding. Both the finding of minor polysomic cell populations and the observation of trisomic and tetrasomic cells together in the same tumor specimens are in agreement with the generally accepted theory that the genetic evolution of solid tumors converges on repeated rounds of tetraploidization and subsequent chromosome loss [24]. Following this concept, minor fractions of cells which show gain of hybridization signals are thought to be a manifestation of the onset of tetraploidization of a tumor [25].

An explanation for the lower frequency of gain for Y could be that relative loss of one Y chromosome in a tetraploid tumor cell will result in monosomy for Y, thereby making this cell indistinguishable from a diploid cell. Simultaneous loss and gain of a chromosome in the same specimen, indicative for heterogeneous changes, was rare for chromosomes 1 and 18 (respectively, one and two cases) and occurred in 16% of the cases investigated with the Y probe.

The correlation between DNA-FCM and FISH was less strong than expected. Surprisingly, most DNA diploid tumors showed significant gain of one or more of the investigated chromosomes. Apparently, already in diploid tumors additional copies of especially chromosome 18 could be present without being detected by DNA-FCM. On the other hand, an aneuploid DNA histogram did not imply the presence of numerical aberrations for all three investigated chromosomes. However, as expected, with an increasingly aberrant and complex histogram, more of the investigated chromosomes became aberrant.

The occurrence of numerical aberrations in the three investigated chromosomes was independent of tumor grade or stage, nor was there a correlation between the presence of aberrations in each individual chromosome and grade or stage (not shown). However, as expected, the DNA-FCM of a tumor was more likely to be abnormal with increasing grade and stage. At present two other studies report the detection of numerical chromosomal aberrations with centromere-specific probes in PC [26, 27]. The first study investigated chromosomes 7, 17, X, and Y on paraffin sections. Although this method will lead to an underestimation of the number of in situ signals, due to the loss of nuclear material [28], aberrations were found in 5/11 investigated tumors. These tumors were all of advanced stage or grade with a large tumor volume. No details were presented on specific chromosomal aberrations found, but some heterogeneity with respect to different regions in the tumor area was reported. The other study used cells obtained from tissue cultures of 10 PC specimens. The copy numbers of chromosomes 7, 8, 10, 16, 17, and 18 were assessed by FISH. Frequent losses and gains were observed for all chromosomes investigated. However, culturing artifacts, caused by preferential growth of subgroups of tumor cells, or even normal cells [8], cannot be ruled out. Unfortunately, as demonstrated by the high percentages of nuclei with 0 and 1 spot, the specificity of the hybridizations was rather low, making it difficult to draw any definite conclusions from these data.

In bladder carcinoma, FISH studies [25, 29] have shown numerical aberrations for chromosome 1 in 20–25% of the cases, and also in breast cancer aberrations of chromosome 1 were shown to occur [30]. Cytogenetic studies on PC have shown that chromosome 1, when aberrant, is most frequently structurally rearranged [3, 4, 31]. In PC cell lines, gain of chromosome 1 is also common [32–36]. The relatively common gain of chromosome 1 we report here is in agreement with these results.

Cytogenetically, chromosome 18 was not shown to be nonrandomly or specifically affected in PC, but in two LOH studies, loss of loci on the long arm of chromosome 18 was reported in 3/7 [15] and 2/12 [16] informative cases. Our data showed that complete loss of one homolog was infrequent, but that gain was common. Chromosomal duplication, driven by gene loss, could be a possible mechanism behind this observation.

Loss of Y is a recurrent chromosomal aberration in PC [1–3, 5, 17], sometimes found as the sole anomaly. Also with in situ hybridization on paraffin sections some PCs were shown to have loss of Y [27, 37]. The frequency of 53% we found in the present study is quite high, but could be biased by the low cut-off percentage derived from the studies in BPH specimens, which served as a control. The loss of Y has been described, not only in other solid tumors but also in benign and normal tissues. So, on the other hand, loss of the Y chromosome may also reflect a general state of tissue hyperproliferation and as such may not exclusively be related to neoplastic processes.

The finding that gain of chromosomes 1 and Y was restricted to DNA aneuploid tumors is not surprising. However, we have no explanation for the almost consistent finding of a higher percentage of disomic (monosomic for Y) cells in aneuploid tumors than was to be expected on the basis of DNA ploidy. Although applied on the same isolated cells, the sensitivity of the techniques appears to be different. By FISH the percentage of aneusomic nuclei seldom

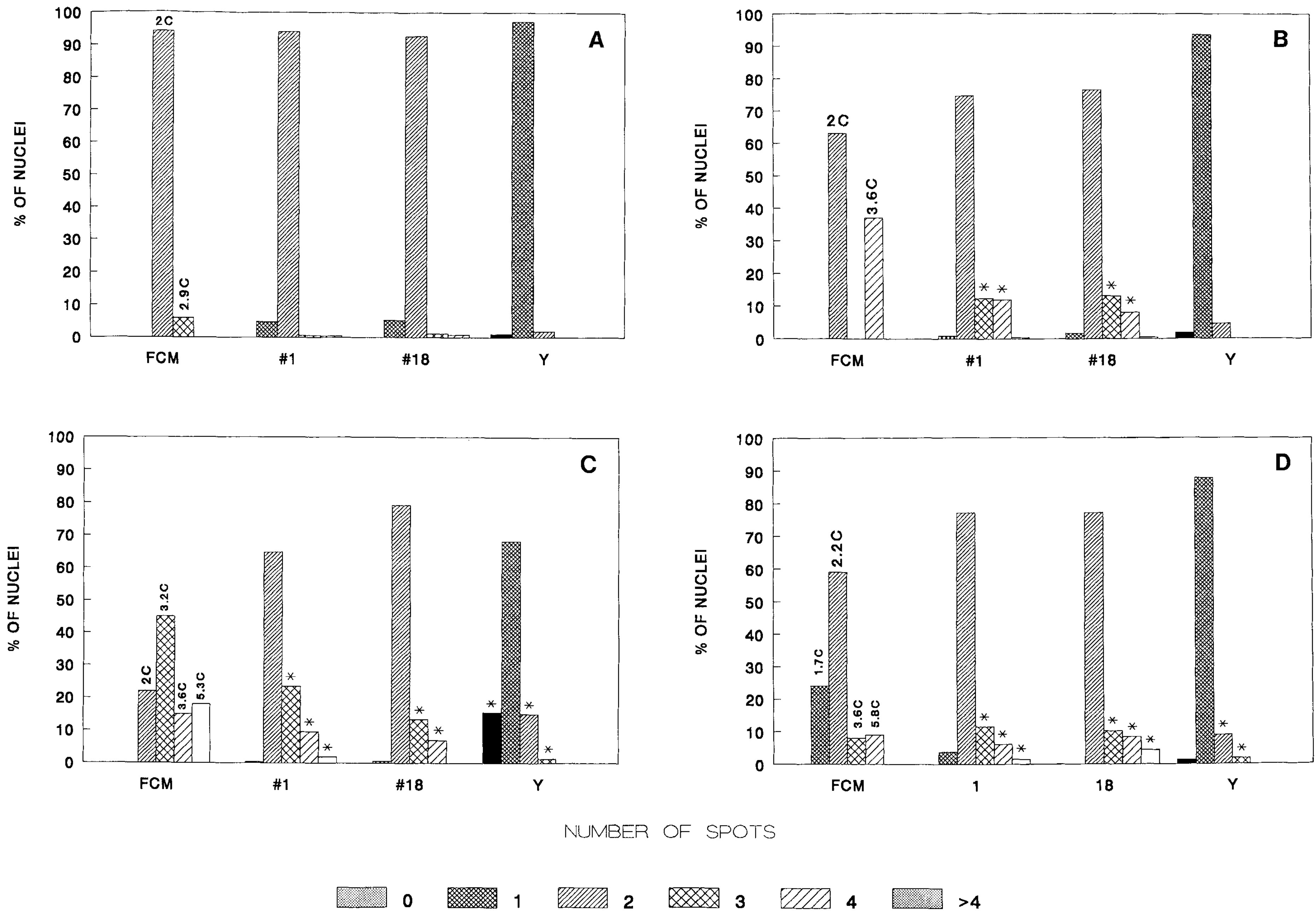

Fig. 2.

reached 30%, while with DNA-FCM the percentage of aneuploid nuclei could be over 90%. This phenomenon was observed before in PC and bladder carcinoma in comparative studies using FISH and DNA-FCM [8, 22, 29]. A similar effect has been described recently in a comparative study of cytogenetic and DNA-FCM data on bone and soft tissue tumors [38]. In karyotypically abnormal tumors which were aneuploid, a tendency towards DNA indices higher than the chromosomal index was reported. This was partly explained by the assumption that in these tumors, which often had complex chromosomal rearrangements, the size of some marker chromosomes probably exceeded the size of normal homologs. This could also be the case for PCs which show moderate differences, but still cannot explain the large discrepancies found in some highly aneuploid tumors.

The correlation between tumor ploidy and tumor aggressiveness has been well established [9–12]. Recently, Lundgren et al. [31] showed the association of the presence of clonal chromosomal aberrations with an unfavorable outcome in PC patients. In the present study we found that the presence of numerical aberrations of the chromosomes we investigated was unrelated to tumor grade or stage.

In conclusion, the results obtained with FISH of only three centromere-specific probes show significant aneuploidy in small subpopulations in a total of about 90% of the investigated tumors. This is in contrast with FCM where 63% of the tumors show an aneuploid peak. Both the results obtained with FISH and with FCM indicate that cytogenetic studies of PC after short-term tissue culture selectively have produced karyotypic data on the normal cell component of the tumor. Therefore, to gain more insight into the cytogenetic composition of PC, FISH studies of all relevant (parts of) chromosomes appears for the time being the technology of choice.

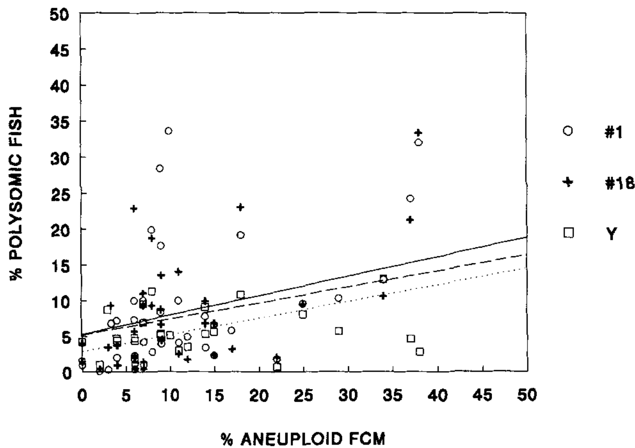

Fig. 3. Correlation between percentage aneusomy by FISH and percentage aneuploidy by FCM. Regression analysis. ○, chromosome 1; +, chromosome 18; □, Y. Regression lines: solid line, chromosome 1; hatched line, chromosome 18; dotted line, Y.

TABLE IV. Relationship Between Stage, Numerical Aberrations, and Ploidy

Stage	No.	% Aberration (FISH)	% Aberration (FCM)
T1	0	—	—
T2	7	86	43
T3	16	88	63
T4	19	84	79

TABLE V. Relationship Between Grade, Numerical Aberrations, and Ploidy

Grade	No.	% Aberration (FISH)	% Aberration (FCM)
1	5	80	60
2	16	88	50
3	21	86	81

Fig. 2. Examples of spot distribution of chromosomes 1, 18, and Y and FCM analysis in **A:** a DNA diploid tumor (PC352); **B:** a DNA tetraploid tumor (PC355); **C:** a tumor with multiple aneuploid stemlines (PC290); and **D:** a DNA hypodiploid tumor (PC269). * = above cut-off value.

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