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Interphase Cytogenetic Analysis of Prostatic Carcinomas by Use of Nonisotopic *in Situ* Hybridization[1]

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ABSTRACT

To gain a better understanding of chromosomal aberrations in direct correlation with histology, we studied tumor material from 35 patients (36 regions) with primary prostate carcinoma by nonisotopic *in situ* hybridization. Nine biotinylated DNA probes were used on serial paraffin sections (centromer-specific probes for X, Y, 1, 7, 8, 10, 17, and 18, and a telomer-specific probe for 1p; ONCOR). Of the 324 hybridized sections, 94% were suitable for evaluation. In 34 of the 35 cases (35 of 36 regions) 1–8 chromosomal aberrations were detected. Chromosome X showed supernumerary centromer copies in 44% of cases. The probes for chromosomes 1, 1p, 10, and 18 demonstrated deletions in 25, 23, 40 and 58% of cases, respectively. Gains as well as deletions were present for Y, 7, 8, and 17 in 31, 25, 36, and 58% of cases, respectively. In 27% of cases discordant copy numbers of the centromer- and the telomer-specific probes for chromosome 1 were observed. No aberration which might be specific for prostate cancer could be established. The rate of aneusomy increased significantly with histological grade. Intratumoral heterogeneity of chromosomal aberrations was revealed in one case. Due to the higher sensitivity of nonisotopic *in situ* hybridization, aneusomic cases outnumbered cases with cytometrically determined DNA aneuploidy. In view of published results of metaphase preparations, the high frequency of aneusomy and some of the chromosomal aberrations detected by nonisotopic *in situ* hybridization were unexpected.

INTRODUCTION

Carcinoma of the prostate is the most common malignancy in men in the United States of America and in Western Europe (1). Prostatic cancer is unique among human malignancies, because its prevalence at autopsies by far exceeds clinically manifest carcinomas worldwide (1). This uniformly high prevalence rate contrasts with the widely varying clinical incidence and mortality rate of prostatic carcinoma in different countries. Evidence is increasing that alterations in genomic DNA ploidy may influence the prognosis of patients with prostatic cancer. Several studies have shown that DNA ploidy determined either by means of FCM[4] (2–4) or ICM (5) is an independent prognostic parameter in prostatic carcinoma (6), because DNA-diploid tumors progressed less frequently than aneuploid cases. Distinct chromosomal aberrations, however, cannot be detected by means of FCM or ICM. Thus, other methods providing additional information on genomic changes would be useful.

Karyotyping of prostatic carcinomas is impeded by the relatively low proliferative activity of the tumor cells and the difficulty of obtaining a sufficient number of metaphases for chromosome analysis. Therefore, only about 200 prostatic carcinomas have been studied by means of traditional cytogenetics up to now (7–10). In a recent karyotyping study of 30 primary prostatic carcinomas the most common karyotype seen was a normal diploid karyotype (8). Breitkreuz *et al.* (10), however, demonstrated that one-third of biopsies from patients with prostatic cancer used for karyotyping contained no tumor tissue in histological control sections. This finding points to another obstacle of traditional cytogenetic works, which is the lack of a direct morphological correlation between the analyzed metaphases and the original cells. Furthermore, minor tumor cell clones or non-tumorous stromal cells might overgrow *in vitro* and falsify the results. The same is basically true for molecular biological approaches that use tissue extracts for DNA blotting. Like for most other solid malignant tumors no single chromosomal aberration which might be specific for prostatic carcinoma has yet been identified. Nevertheless, Sandberg (7) recently summarized the literature on cytogenetic findings and proposed a scheme with a number of ordered genetic events which might be involved in the development of prostatic cancer in accordance with the concept of multistep carcinogenesis (11).

In order to gain more insight into chromosomal aberrations of prostatic carcinomas in close correlation with morphology, we studied prostatic carcinomas by means of NISH by using 8 biotinylated centromer-specific DNA probes and one telomer-specific probe. This technique allows us to enumerate specific chromosomes or chromosome parts within interphase nuclei, not only in cell cultures and touch preparations of fresh tumor material but also in tissue sections of formalin-fixed and paraffin-embedded tumor material (12–14). The available data in the literature concerning chromosomal *in situ* hybridization on prostatic cancer are scarce and mostly restricted to a small number of cases and/or few DNA probes (10, 15–17). We developed an improved protocol for the application of chromosomal NISH on prostatic carcinomas. To the best of our knowledge, ours is the first study that applies NISH with the use of a panel of chromosome-specific DNA probes on a considerable number of prostatic carcinomas, and correlates the results with histology and with the ploidy status of the tumor cells determined by means of FCM and/or ICM.

MATERIALS AND METHODS

Formalin-fixed and paraffin-embedded tumor tissue from 35 patients with primary carcinoma of the prostate (23 prostatectomy specimens, 12 transurethral resections; mean age of the patients, 68.2 ± 10.3 years) were studied. Within the specimens regions suitable for NISH were selected in routinely hematoxylin and eosin-stained sections (4 μm thick).

The cases were revised and graded according to the Gleason scheme (18) and simultaneously to the classification of the German Pathological-Urological Study Group (19). Because the selected areas were histologically homogeneous, the Gleason score was restricted to a single grade ranging from 1 to 5. In the German classification the MG is determined by a score that combines the degree of histological differentiation (well differentiated glandular, 0; poorly differentiated glandular, 1; cribriform, 2; solid/trabecular, 3) with the grade of nuclear anaplasia (low, 0; moderate, 1; high, 2). The total score is 5; 0 and 1 points refer to grade 1; 2 and 3 points refer to grade II; and 4 and 5 points refer to grade III. Furthermore, each grade can be subdivided into "a and b" depending, for example, on whether grade II has 2 or 3 points. The mean nuclear diameters were 8.3 ± 0.9 μm in non-neoplastic prostatic epithelium, and 9.2 ± 1.0, 9.9 ± 1.2, and 10.4 ± 1.2 μm in carcinomas with MG I, II, and III, respectively. In one case regional differences in histological differentiation of carcinoma were present. These areas were analyzed separately in order to

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[4] The abbreviations used are: FCM, flow cytometry; ICM, image cytometry; NISH, nonisotopic *in situ* hybridization; MG, grade of malignancy; PBS, phosphate-buffered saline; FISH, fluorescence *in situ* hybridization.

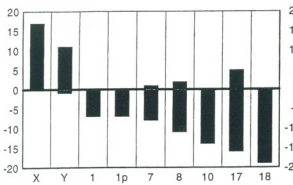

Fig. 1. All chromosomal aberrations detected in serial sections of 36 regions from 35 prostatic carcinomas by means of interphase cytogenetics with the use of 9 chromosome-specific DNA probes.

2 probes, respectively, and in 2 cases of 3 chromosomal DNA probes was not possible either due to suboptimal morphological quality of the tissue after processing or lack of tumor tissue in the deeper serial sections, *i.e.*, 306 of 324 (94%) hybridized paraffin sections could be studied.

With higher grade carcinoma (determined either according to the German classification or to the Gleason scheme) the rate of chromosomal aneusomy increased significantly (Figs. 2 to 4). In the case with the intratumoral differences in nuclear grading, discrepancies in chromosomal *in situ* hybridization were also revealed, *i.e.*, one aberration in a highly differentiated area (MG Ib) and four aberrations in the moderately differentiated part of the tumor (MG IIa; case 6, Table 1). In 4 other tumors (2 moderately and 2 poorly differentiated carcinomas), remarkable variations of NISH signal counts of 1–3 DNA probes were observed (Fig. 4*d*). With a higher number of enumerated nuclei, however, these variations did not exceed the limits for aneusomy mentioned before.

Besides the normal centromeric copy numbers, gains as well as deletions were observed in the tumor cell nuclei for chromosome probes Y, 7, 8, and 17. A loss of the signal for chromosome Y in carcinoma cells, however, was detected only in one case (Figs. 1 and 4).

For chromosome X either normal or increased NISH signal numbers were revealed, using the centromer-specific DNA probe. A supernumerary X-chromosome was also the most frequent chromosomal gain in our study. On the other hand, some chromosomal probes demonstrated deletions only when the NISH signal numbers were not within the normal range, *i.e.*, DNA probes for 1, 1p, 10, and 18 (Fig. 1). In 15 of the 26 regions where hybridization signals for both the centromeric- and p-telomeric region of chromosome 1 could be enumerated, the signals for both probes were disomic. In 3 cases both probes exhibited a monosomic pattern indicating a concordant deletion, but in 7 cases (27%) discordant copy numbers were observed. In 4 of these cases one copy of the centromer of chromosome 1 was missing, and in 3 cases one copy of the telomer (Fig. 5).

Cytometric Ploidy Status of Prostatic Carcinomas (FCM and ICM). Fifteen of 35 (43%) regions equivalent to the NISH areas were DNA diploid, whereas 20 (57%) exhibited an aneuploid DNA content in the image cytometric analysis of the paraffin sections. The results

Table 1 *Histopathological data, results of interphase cytogenetics, and cytometrically determined DNA ploidy status in 36 tumor regions from 35 prostatic carcinomas*

				Interphase cytogenetics/NISH										
Case	Mat.[a]	Gleas.	MG	X	Y	1	1p	7	8	10	17	18	$\sum$	Ploidy
1	RP	2	Ib	0	0	NE	0	0	0	0	0	NE	0	Di.
2	TUR	2	Ib	0	0	0	0	0	0	0	0	–	1	Di.
3	RP	2	Ib	0	0	0	0	0	0	0	0	–	1	Di.
4	RP	2	Ib	0	0	0	0	0	0	0	–	0	1	Non-di.
5	RP	4	Ib	0	0	–	0	0	0	0	0	0	1	Non-di.
6a[b]	TUR	2	Ib	+	0	0	0	0	0	0	0	0	1	Di.
7	TUR	3	IIa	0	0	NE	NE	0	0	–	0	0	1	Di.
8	RP	4	IIb	0	0	0	0	0	0	–	0	0	1	Non-di.
9	RP	2	IIa	0	0	0	0	0	0	0	–	0	1	Di.
10	RP	2	Ib	0	0	NE	0	0	0	–	0	–	2	Di.
11	RP	3	IIb	0	0	NE	NE	–	0	NE	0	–	2	Non-di.
12	RP	3	IIb	+	0	0	0	0	0	0	0	–	2	Non-di.
13	TUR	3	IIb	0	+	NE	0	0	0	0	+	0	2	Di.
14	RP	4	Ib	+	0	0	0	0	–	0	–	0	3	Di.
15	RP	2	Ib	0	0	–	–	0	0	0	0	–	3	Di.
16	TUR	2	Ib	0	0	NE	NE	–	–	0	–	NE	3	Non-di.
17	RP	4	IIIb	+	0	0	0	–	0	0	–	0	3	Non-di.
18	RP	3	Ib	+	+	NE	–	0	0	–	0	NE	4	Di.
19	RP	3	Ib	0	0	0	NE	0	–	–	–	–	4	Di.
6b[b]	TUR	2	IIa	+	+	0	0	0	0	0	+	–	4	Di.
20	TUR	4	IIb	+	+	–	0	0	0	0	0	–	4	Non-di.
21	RP	3	IIb	+	+	0	0	0	+	0	+	0	4	Non-di.
22	RP	3	IIb	0	0	0	–	0	+	–	–	0	4	Non-di.
23	RP	3	IIb	+	+	0	–	0	0	0	–	0	4	Non-di.
24	RP	3	IIb	+	+	0	0	0	0	0	–	–	4	Non-di.
25	TUR	4	IIIa	0	0	0	0	0	–	–	–	–	4	Di.
26	RP	5	IIIb	+	+	0	0	0	0	0	+	–	4	Non-di.
27	TUR	4	IIIa	+	+	0	0	0	0	–	0	–	4	Non-di.
28	RP	3	IIa	0	0	–	–	0	–	0	–	–	5	Di.
29	RP	4	IIIa	+	0	NE	NE	–	–	–	–	0	5	Non-di.
30	TUR	5	IIIb	+	–	0	0	+	0	0	+	–	5	Non-di.
31	RP	4	IIIa	0	0	0	NE	–	–	–	–	–	5	Non-di.
32	TUR	4	IIIa	+	+	0	–	0	–	–	0	0	5	Non-di.
33	TUR	5	IIIb	0	0	–	0	–	–	–	–	–	6	NE
34	RP	4	IIIa	0	0	–	0	–	–	–	–	–	6	Non-di.
35	TUR	5	IIIb	+	0	–	–	–	–	–	–	–	8	Non-di.
Total				16	11	7	7	9	13	14	21	19	117	

[a] Mat., material; RP, radical prostatectomy; TUR, transurethral resection; Gleas., Gleason grade (18); 0, no aberration; +, supernumerary signal; –, deletion; NE, not evaluable; $\sum$, sum of chromosomal aberrations per region; Ploidy, determined by ICM on corresponding paraffin sections; Di., diploid; Non-di., nondiploid.

[b] Two regions from one case with different nuclear grading.

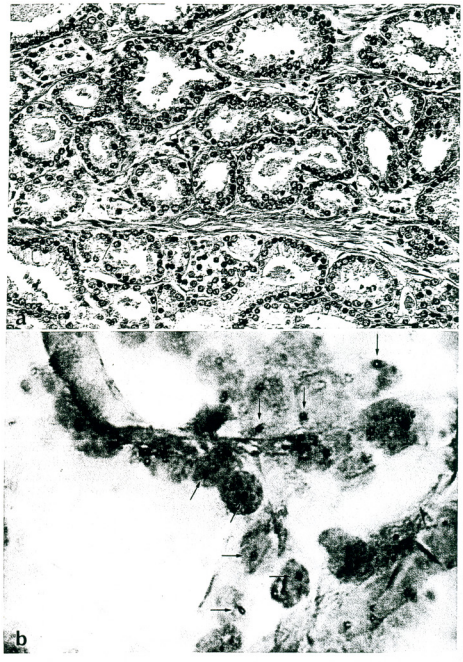

Fig. 3. *a*, highly differentiated prostatic carcinoma (case 4, Gleason 2/MG Ib). H&E, × 16. *b*, showing only a deletion of one chromosome 17 centromer copy (*arrows*) in the interphase cytogenetic analysis (oil immersion, × 100).

accordance with our data, no Y deletion was detected within nonneoplastic prostatic epithelial and stromal cells. These results contradict cytogenetic studies which report that a loss of chromosome Y is frequent in cells obtained from healthy elderly men (7).

A diminished number of NISH signals for chromosome 18 was the most frequent chromosomal loss in our material. In karyotyping studies this deletion has been described previously in only one case (8). Furthermore, losses of NISH signals were observed quite often for chromosomes 8 and 10. Considering molecular biological studies in which allelic losses in prostate cancer were investigated (33, 34), chromosome arms 8p, 10q, 16q, and 18q might well be sites for tumor suppressor genes. Using mostly centromer-specific DNA probes, structural changes in most distal regions of the chromosomes are beyond the scope of our investigation. However, the NISH results give rise to speculations about links to molecular genetic mechanisms of carcinogenesis. The putative colorectal tumor suppressor gene *dcc* is located on the long arm of chromosome 18. Whether *dcc* also participates in prostatic carcinogenesis is still unknown.

A deletion of chromosome 10q has been discussed as a marker for increased metastatic risk in prostate cancer (9), but a distinct metastasis suppressor gene has not yet been identified.

P53, which is regarded as another important tumor suppressor gene for many human malignancies, has been mapped to the short arm of chromosome 17 (35). Loss of heterozygosity for the 17p chromosomal region as reflected in allelic loss could be established recently in a metastatic carcinoma of the prostate (36). *P53* mutations, however, are estimated to occur in no more than 20% of prostatic cancers (37). How often the observed losses of NISH signals for the centromer region of chromosome 17 are related with *P53* deletions, needs to be investigated.

Complementary to the loss of tumor suppressor gene amplifications or over-expressions of oncogenes coding for growth factors or growth factor receptors are known to be involved in cancer initiation and

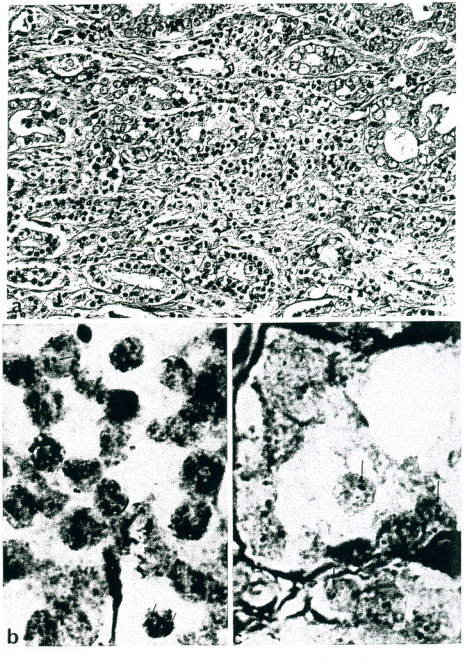

Fig. 5. *a*, moderately differentiated prostatic carcinoma (case 23, Gleason 3/MG IIb). H&E, × 16, with loss of one NISH signal for the 1p-telomer-specific DNA probe; *b*, chromosome 1 centromer-specific probe: 2 signals (*arrows*); *c*, chromosome 1 telomer-specific probe: only one signal in the tumor-cell nuclei (*arrows*; oil immersion, × 100).

It should be emphasized that only relatively small parts of the chromosomal DNA and not the whole chromosomes that are detected with the probes used. Thus, marker chromosomes containing translocated parts of different chromosomes cannot be identified by the applied technique. The use of NISH on paraffin sections, however, has the advantage of clear attribution of the hybridization signals to an individual tumor cell which is not given in metaphase or blotting techniques. A direct correlation with morphology is of particular importance in prostatic cancer, because histological tumor grade proved to be an important parameter for prognosis, and intratumoral heterogeneity is not uncommon (43, 44). In addition, NISH allowed us to study archival material retrospectively, whereas karyotyping requires fresh tumor tissue and is impeded by the frequent failure of prostatic cancer cells to grow and divide *in vitro*. Therefore, only about 200 primary prostatic carcinomas or metastases have been analyzed cytogenetically up to now (7–10), often showing a normal karyotype. The admixture and overgrowth of non-neoplastic cells, which might falsify the results, is difficult to exclude without morphological control.

Table 2 *Correlation between histological/cytological grade of malignancy (MG) and DNA ploidy determined by means of image cytometry on paraffin sections of 35 regions from 34 prostatic carcinomas ($P < 0.01$, χ^2 test)*

	MG I	MG II	MG III	Total
DNA diploid	9	5	1	15 (43%)
DNA nondiploid	3	8	9	20 (57%)
Total	12 (34%)	13 (37%)	10 (29%)	35

As observed previously, the results of FCM and ICM using sections and nuclear suspensions were concordant (45) and DNA ploidy correlated well with histological grading (Table 2). In contrast to this the comparison of NISH and DNA cytometric data showed no significant correlation (Table 3). The low rate of concordance is easily explained by the fact that chromosomal aberrations can be detected by FCM only when they exceed at least 4% of the total DNA amount, *i.e.*,