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# Interphase Cytogenetics of Prostatic Adenocarcinoma and Precursor Lesions: Analysis of 25 Radical Prostatectomies and 17 Adjacent Prostatic Intraepithelial Neoplasias

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Twenty-five radical prostatectomy specimens were screened for the presence of numerical chromosome changes within the adenocarcinoma as well as in 17 adjacent prostatic intraepithelial neoplasias (PIN) by means of interphase in situ hybridization (ISH) to routinely processed tissue sections. To this end a defined alfoid repetitive DNA probe set was used, specific for the centromeres of chromosomes 1, 7, 8, 10, 15, and Y. The cytogenetic information was correlated with histopathological and clinical features as well as with DNA ploidy. Numerical aberrations of at least one chromosome were shown in 13 of 25 cases (52%). Alterations of chromosome 8 and loss of the Y chromosome were the most frequent findings (both 20%), followed by loss of chromosomes 15 (16%) and 10 (12%). Gain of chromosome 7 was seen in 8% of cases. No aberrations of chromosomes 7, 8, 10, and 15 were found in the adjacent PIN lesions, whereas loss of the Y chromosome in both PIN and tumor occurred in two cases. Also, (low level) aneuploidy was observed in 76% of these PIN lesions. Ploidy of the carcinomas as assessed by ISH correlated well with ploidy measured by DNA flow cytometry (FCM; $P < 0.02$). Due to the more specific correspondence between ISH and tumor pathology, pathologic grade correlated with ISH aneuploidy ($P < 0.05$), whereas FCM ploidy did not. Furthermore, genetic heterogeneity within a tumor was seen, as judged by the focal appearance of chromosomal aberrations. Chromosomal alterations occurred in all grades and stages, although loss of chromosome 10, gain of chromosome 7, and aberrations of chromosome 8 tended to predominate in more advanced cancers. *Genes Chromosom Cancer 12:241–250 (1995).*


## INTRODUCTION

In Europe and the United States, prostate cancer is presently the second leading cause of male deaths from malignant neoplasms (Carter and Coffey, 1990). As the population ages it is predicted that the number of patients will increase steadily over the next decade (Carter and Coffey, 1990). The clinical course of prostate cancer is highly variable and unpredictable. Present methods of assessing the prognosis for prostate cancer include clinical staging and histopathological grading (Gleason, 1992; Schröder et al., 1992). Unfortunately, these methods fail to provide consistent predictive information regarding the clinical outcome of an individual tumor, particularly in tumors confined to the prostate. Cellular DNA measurements provided useful information on the biological aggressiveness of the tumor (Deitch and deVere White, 1992). The therapeutic strategy in individual cases, however, is still difficult to design. Hence, there is a need to identify characteristics of prostate tumor cells that would help in defining the biological aggressiveness of individual tumors and guide the choice of therapy. An understanding of prostate cancer cytogenetics might provide such information.

Knowledge of cytogenetic alterations in prostate cancer is relatively sparse when compared with other common malignancies, and a consistent primary cytogenetic change has yet to be identified (Sandberg, 1992). In general, cytogenetic studies of prostate cancer by karyotyping of metaphase cells are hampered by preferential growth of normal (diploid) cells and by the low mitotic index of the tumor cells. Conventional cytogenetic analyses have revealed loss of the Y chromosome, trisomy of chromosome 7, and loss of 7q, 8p, and 10q chromosome arms (Lundgren et al., 1988, 1992a; Brothman et al., 1990, 1991; Micale et al., 1992; Sandberg, 1992; Arps et al., 1993). Allelotyping of prostate carcinoma using restriction fragment length polymorphism (RFLP) showed allelic losses on the 8p, 10p, 10q, 16q, and 18q arms (Carter et al., 1990; Bergerheim et al., 1991; Kunimi et al., 1991; Bova et al., 1993; Chang et al., 1994).

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Prostatic intraepithelial neoplasia (PIN) is characterized by cytological abnormality and proliferation of the normal luminal cell layer lining prostatic ducts and acini. PIN occurs more often in prostates with invasive carcinoma than in those without (reviewed by Brawer, 1992). In biopsy specimens containing high grade PIN, the patient usually develops clinically invasive cancer within a few years (Bostwick and Brawer, 1987; Weinstein and Epstein, 1992). In general, PIN lesions are considered to be the precursors of prostatic adenocarcinoma. DNA quantitation of isolated PIN lesions by flow cytometry (FCM) showed aneuploidy in about 40% of cases (Crissman et al., 1993). As far as we know, no karyotyping data are available on PIN lesions. However, Macoska et al. (1993) found focal loss of chromosome Y in one of two PIN lesions by performing fluorescence in situ hybridization (FISH) analysis.

Cytogenetic analyses based on dissociated tissue eliminate the morphological architecture of the tissue. In this situation it is difficult to correlate specific chromosomal changes with histological characteristics of the source cells. In the last decade, non-isotopic in situ hybridization (ISH) with (peri)-centromeric chromosome specific DNA probes has emerged as a powerful tool for the discrimination of numerical chromosome changes in interphase cells of solid tumor specimens (Cremer et al., 1988; van Dekken et al., 1990a,b; Persons et al., 1993). This technique has recently been adapted for application to tissue sections, thereby allowing combined cytogenetic and histologic analysis (Hopman et al., 1991; van Dekken et al., 1992; Wolman et al., 1992; Kim et al., 1993; Macoska et al., 1993; van Dekken et al., 1993; Krishnadath et al., 1994).

In this study we have applied ISH to paraffin-embedded tissue sections of 25 primary prostate adenocarcinomas with adjacent dysplasias (PIN). To our knowledge this is the largest panel of prostatic tumors and PIN lesions examined by this method. The following specific questions were addressed: 1) Can the reported numerical chromosomal changes in prostatic adenocarcinoma be confirmed? 2) Can new chromosomal aberrations be identified? 3) Do preneoplastic lesions adjacent to tumors contain (the same) cytogenetic aberrations? 4) Are specific chromosomal changes in prostatic adenocarcinoma associated with more aggressive tumor behavior? For this purpose, we used a set of relevant (peri)centromeric alfoid DNA probes, specific for chromosomes 1, 7, 8, 10, 15, and Y. The centromeric probes for chromosomes 1 and 15 were added to this panel, since in solid cancers a gain of chromosome 1 is often seen (Atkin, 1986). Furthermore, loss of chromosome 15 was seen by us in cancers of the bladder (Schervish and van Dekken, in preparation). In addition, ploidy status of the tumor was examined by DNA FCM.

## MATERIALS AND METHODS

### Tissue Specimens

Routinely processed, formalin-fixed, paraffin-embedded materials, obtained between 1990 and 1992, from radical prostatectomies of 25 patients with primary prostatic adenocarcinoma were used for this study. Tumors were staged according to the TNM classification (Schröder et al., 1992) and graded according to the Gleason system (Gleason, 1992). The Gleason grading system recognizes five growth patterns with increasing loss of histological differentiation. Forty-one Gleason areas were discriminated in our panel of 25 prostatic tumors. Seventeen tumors were accompanied by PIN lesions.

### ISH

ISH with the biotin-labeled DNA probe set, specific for chromosomes 1, 7, 8, 10, 15, and Y, was performed as described by van Dekken et al. (1992, 1993). Briefly, to facilitate DNA probe accessibility to the cellular DNA, sections were digested with 0.4% pepsin (Sigma, St. Louis, MO) in 0.2 M HCl at 37°C for 5–30 min (mean: 14 min). Cellular DNA was heat denatured for 2 min in 70% formamide in 2× SSC (pH 7.0); the chromosome specific repetitive DNA probes were denatured for 5 min at 70°C in a hybridization mixture containing 1–2 μg/ml probe DNA, 500 μg/ml sonicated herring sperm DNA (Sigma), 0.1% Tween-20, 10% dextran sulfate, and 60% formamide in 2× SSC at pH 7.0. The slides were then incubated overnight at 37°C in a moist chamber and subsequently washed. Histochemical detection of the biotinylated DNA probes was performed by the standard avidin-biotin complex (ABC) procedure and immunoperoxidase staining. Sections were counterstained with hematoxylin.

### Evaluation of ISH Results

The DNA probe set was analyzed for each prostate adenocarcinoma on consecutive 4 μm sections in a previously defined tumor area with a certain Gleason score. A section size of 4 μm was chosen after evaluating the degree of nuclear overlap (= countability) and section thickness. For each of the probes, 100 "intact" (= spherical) and non-overlapping 4 μm nuclear slices were counted by 2 independent investigators (100 nuclei each), and

the number of solid diaminobenzidine (DAB) spots per nuclear fragment was scored (0, 1, 2, 3, 4, >4 spots per nuclear slice). The individual DNA probe spot distributions were then compared and totaled, when no significant counting differences between the investigators were found. In case a numerical aberration was detected, a third independent investigator was consulted. The probe spot distributions were statistically evaluated by means of the Kolmogorov-Smirnov test (Young, 1977). Underrepresentation of a specific chromosome was seen as a shift to the left of the DNA probe distribution, when compared with non-aberrant probe distributions. Conversely, gain of a specific chromosome was seen as a shift to the right. This method is described in detail in previous studies (van Dekken et al., 1992, 1993). Chromosome 1 was used as a measure for aneuploidy, since no isolated aberrations were found for this probe. Furthermore, in each case the non-aberrant probes revealed identical ploidy patterns for the Gleason areas.

On each tissue section leukocytes, benign prostatic hyperplasia (BPH), nerve cells, etc., served as internal controls to evaluate the quality of ISH and to detect probe polymorphisms. Internal controls (normal prostate glands: 13 cases; BPH: 4 cases; leukocytes: 13 cases; other cells: 5 cases) on the same tissue sections always showed a diploid pattern (van Dekken and Alers, 1993; Krishnadath et al., 1994). The number of nuclei with a hyperdiploid spot number (likely artifacts) in these internal controls never exceeded 2.5%. This is illustrated by case 3. In Figures 1A and 1B the diploid probe spot pattern for 4 μm tissue sections is shown. Due to sectioning, the normal control cells generally displayed 0 or 1 spot for the autosomes in 10% and 40% of nuclei, respectively. Moreover, in case 3 chromosomal aberrations (loss of Y and loss of chromosome 15) and aneuploidy are also demonstrated in the tumor areas (Fig. 1C,D). Despite the 4 μm sectioning artifact, which results in truncated nuclei, specific chromosome aberrations were detected and could be statistically evaluated. In contrast with true loss of chromosome 15, polymorphism for chromosome 15 might have been considered to be an aberration in four tumor specimens if no internal controls had been examined. In these cases the alpha satellite DNA probe showed strong polymorphism in both tumor cells and control cells.

### DNA FCM

DNA content of the paraffin material was measured as described by Hedley et al. (1983). Three to five approximately 25–50 μm slices of Gleason-graded tumor cell areas were selectively cut out of the paraffin blocks. The lower boundaries were then examined for presence of tumor and pathologic grade. Correspondence between upper and lower boundary was seen in 93% of the tumor samples. Only in 3 of 41 (7%) areas the tumor area was not present at the lower boundary. FCM and analysis of the ethidium bromide (Sigma)-stained nuclei from these areas was performed using a FACScan (Becton Dickinson, Mountain View, CA). Tissue from a normal prostate served as a diploid control. A DNA index between 0.8 and 1.2 was considered diploid.

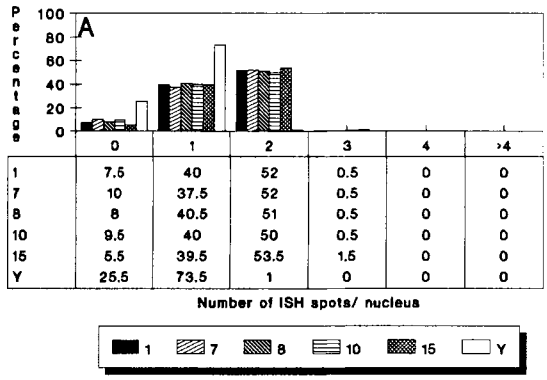
## RESULTS

The results of ISH, histopathological examination (Gleason grading), staging, and DNA FCM are summarized in Table 1. ISH revealed numerical aberrations of at least 1 examined chromosome in 13 of 25 cases (52%): Loss of the Y chromosome and both loss and gain of chromosome 8 were the most common findings (20%), followed by loss of chromosomes 15 (16%) and 10 (12%), and gain of chromosome 7 (8%).

Loss of chromosome 8 was seen in two patients and gain of chromosome 8 in three patients. To illustrate heterogeneity, in case 4 loss of chromosome 8 was seen in the Gleason 3 area only (Fig. 2A, Table 1). In case 19 loss of the Y chromosome was seen in both Gleason areas (Fig. 2B). Loss of chromosome 15 was seen in three patients. In case 25 loss of chromosome 15 was seen in anaplastic areas (Fig. 2C). Loss of chromosome 10 was observed twice in cribriform growth patterns (cases 9 and 13) and once in a high grade tumor (case 22; Fig. 2D,E). In the latter tumor a gain of chromosome 7 was seen in the Gleason 5 area (Fig. 2E,F). No aberrations of chromosome 1 were found. A lymph node metastasis of case 2, as well as the primary tumor, showed no chromosomal aberrations for this probe set. For all cases no chromosome abnormalities were seen in normal prostatic epithelium and BPH.

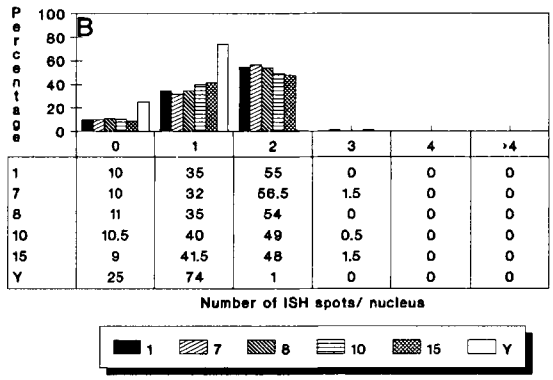
Generally, FCM data corresponded well with ISH ploidy (Spearman's rank correlation coefficient $r_s = 0.5219$: $P < 0.02$). In nine cases ISH revealed differences in ploidy within a tumor that were not detected by FCM. Seventy-three percent of the 41 Gleason areas (80% of the 25 tumors) showed a varying rate of hyperdiploidy for chromosome 1, ranging from 2.5 to 42.5%. This rate of aneuploidy as detected by ISH increased with

Case 3: LEUKOCYTES



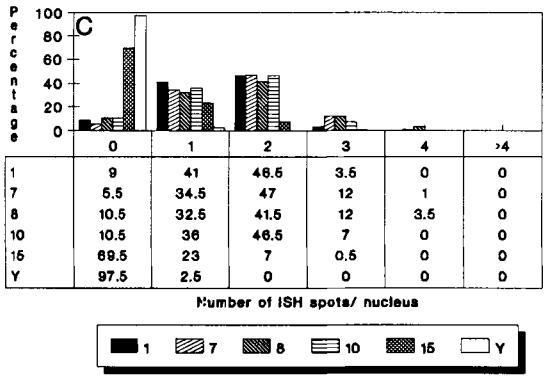

| | 0 | 1 | 2 | 3 | 4 | >4 |
|---|---|---|---|---|---|---|
| 1 | 7.5 | 40 | 52 | 0.5 | 0 | 0 |
| 7 | 10 | 37.5 | 52 | 0.5 | 0 | 0 |
| 8 | 8 | 40.5 | 51 | 0.5 | 0 | 0 |
| 10 | 9.5 | 40 | 50 | 0.5 | 0 | 0 |
| 15 | 5.5 | 39.5 | 53.5 | 1.5 | 0 | 0 |
| Y | 25.5 | 73.5 | 1 | 0 | 0 | 0 |

Case 3: HYPERPLASIA



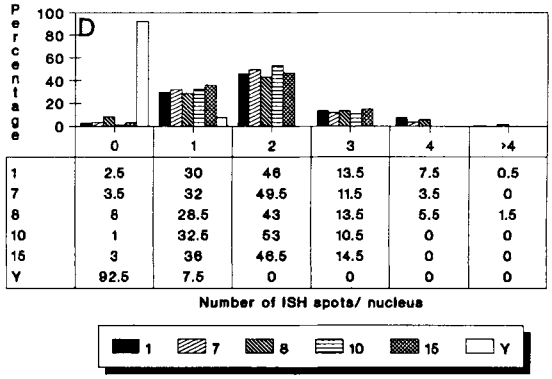

| | 0 | 1 | 2 | 3 | 4 | >4 |
|---|---|---|---|---|---|---|
| 1 | 10 | 35 | 55 | 0 | 0 | 0 |
| 7 | 10 | 32 | 56.5 | 1.5 | 0 | 0 |
| 8 | 11 | 35 | 54 | 0 | 0 | 0 |
| 10 | 10.5 | 40 | 49 | 0.5 | 0 | 0 |
| 15 | 9 | 41.5 | 48 | 1.5 | 0 | 0 |
| Y | 25 | 74 | 1 | 0 | 0 | 0 |

Case 3: GLEASON 2 AREA




| | 0 | 1 | 2 | 3 | 4 | >4 |
|---|---|---|---|---|---|---|
| 1 | 9 | 41 | 46.5 | 3.5 | 0 | 0 |
| 7 | 5.5 | 34.5 | 47 | 12 | 1 | 0 |
| 8 | 10.5 | 32.5 | 41.5 | 12 | 3.5 | 0 |
| 10 | 10.5 | 36 | 46.5 | 7 | 0 | 0 |
| 15 | 69.5 | 23 | 7 | 0.5 | 0 | 0 |
| Y | 97.5 | 2.5 | 0 | 0 | 0 | 0 |

Case 3: GLEASON 3 AREA




| | 0 | 1 | 2 | 3 | 4 | >4 |
|---|---|---|---|---|---|---|
| 1 | 2.5 | 30 | 46 | 13.5 | 7.5 | 0.5 |
| 7 | 3.5 | 32 | 49.5 | 11.5 | 3.5 | 0 |
| 8 | 8 | 28.5 | 43 | 13.5 | 5.5 | 1.5 |
| 10 | 1 | 32.5 | 53 | 10.5 | 0 | 0 |
| 15 | 3 | 36 | 46.5 | 14.5 | 0 | 0 |
| Y | 92.5 | 7.5 | 0 | 0 | 0 | 0 |

Figure 1. DNA probe frequency distributions of the number of hybridization spots per nucleus for case 3 after ISH with the DNA probe set, specific for chromosomes 1, 7, 8, 10, 15, and Y, to 4 μm tissue sections. For all of the probes 100 spherical and non-overlapping nuclei were counted each by 2 independent investigators. The results were added and plotted as a percentage per probe. **A:** Leukocytes of patient 3 showing a diploid ISH profile. No aberrations are seen. **B:** BPH, also displaying the diploid ISH profile for all probes. **C:** Gleason 2 area of the tumor showing loss of the Y chromosome and loss of chromosome 15, indicated by a shift to the left of the DNA probe distribution. **D:** Gleason 3 area of this tumor revealing loss of the Y chromosome only.

higher Gleason grades for both area and total score (Spearman's rank correlation coefficient $r_s = 0.3197$ and $r_s = 0.4241$, respectively: both $P < 0.05$), whereas no statistically significant correlation was found between Gleason score and FCM ploidy.

In both low grade (Gleason score $\leq 6$) and high grade (Gleason score $\geq 7$) tumors approximately the same number of chromosomal aberrations were seen (Table 2). However, the type of chromosomal aberration seemed to differ between low and high grade tumors: Although loss of chromosomes 15 and Y occurred in all Gleason patterns, gain of chromosome 7, alterations of chromosome 8, and loss of chromosome 10 were seen predominantly in the higher pathologic grades (Table 2). A comparable percentage of chromosomal aberrations was found both in tumors that were confined to the prostate (T2 tumors) and in tumors that invaded the prostatic capsule or other organs (T3 and T4 tumors; Table 3). Here also loss of chromosomes 15 and Y was observed irrespective of stage, whereas loss of chromosome 10, gain of chromosome 7, and aberrations of chromosome 8 were noted in T3 and T4 tumors only (Table 3).

In 17 tumors (high grade) PIN lesions adjacent to the tumor cells were analyzed. No numerical aberrations were found of chromosomes 7, 8, 10, and 15 (Table 4, Fig. 3A,B). In cases 3 and 19, however, loss of the Y chromosome was observed in both adenocarcinoma and PIN lesions (Table 4, Fig. 3C,D). In the other three cases with loss of the Y chromosome in tumor glands, no loss of Y

TABLE 1. Clinical Data of Patients and Results of Pathological Examination, ISH, FCM

| Case | Age (years) | Grade | Types[a] | Stage[b] | FCM[c] | ISH | |
|---|---|---|---|---|---|---|---|
| | | | | | | Aneuploidy[d] | Aberrations* |
| 1 | 69 | 4 | G2 | pT3N0 | D | ± | −Y |
| 2 | 61 | 5 | G2 | T4N2[e] | D | + | |
| | | | G3 | | | ± | |
| 3 | 58 | 5 | G2 | T2N0 | D | ± | −Y, −15 |
| | | | G3 | | | ++ | −Y |
| 4 | 66 | 5 | G2 | pT3N0 | D | + | |
| | | | G3 | | A | + | −8 |
| 5 | 51 | 5 | G2 | pT2N0 | T | +++ | |
| | | | G3 | | | ± | |
| 6 | 63 | 5 | G2 | pT2N0 | D | ns | |
| | | | G3 | | T | ns | |
| 7 | 60 | 6 | G3c | pT2N0 | T | + | |
| 8 | 49 | 6 | G3c | pT3N0 | D | ns | −15 |
| 9 | 67 | 6 | G3c | pT3N0 | D | ± | −10 |
| 10 | 63 | 6 | G3 | pT3N0 | D | ns | |
| 11 | 59 | 7 | G3 | pT3N0 | D | ns | |
| | | | G4 | | T | + | |
| 12 | 55 | 7 | G3 | pT3N0 | T | ns | |
| | | | G4 | | | + | |
| 13 | 53 | 7 | G3c | pT3N0 | A | ++ | −10 |
| | | | G4 | | T | +++ | |
| 14 | 59 | 7 | G3 | pT3N0 | D | ns | |
| | | | G4 | | D | ns | |
| 15 | 70 | 7 | G3 | pT3N0 | D | ns | |
| | | | G4 | | | +++ | |
| 16 | 57 | 7 | G3c | pT4N0 | T | +++ | +8 |
| | | | G4 | | | ± | |
| 17 | 51 | 8 | G3 | pT3N0 | T | +++ | +7, +8 |
| | | | G5 | | A | +++ | |
| 18 | 63 | 8 | G3 | pT4N0 | D | ns | |
| | | | G5 | | D | ns | −Y |
| 19 | 47 | 8 | G3c | pT3N0 | A | ++ | −Y |
| | | | G5 | | | ++ | −Y |
| 20 | 49 | 8 | G4 | pT3N1 | T | +++ | |
| 21 | 64 | 9 | G4 | pT3NO | D | ± | |
| | | | G5 | | | ± | |
| 22 | 67 | 9 | G4 | pT4N0 | D | +++ | −10 |
| | | | G5 | | T | +++ | −10, +7, +8 |
| 23 | 69 | 10 | G5 | pT2N0 | T | +++ | −15 |
| 24 | 65 | 10 | G5 | pT3N0 | D | ++ | |
| 25 | 60 | 10 | G5 | pT3N0 | T | + | −8, −15, −Y |

[a]Dominant Gleason growth pattern(s).
[b]TNM classification: all tumors MO (No distinct metastasis).
[c]D(iploid): 4C peak ≤ 10%; T(etraploid): 4C peak > 10%; A(neuploid): other peak (non-4C).
[d]All control cells revealed <2.5% hyperdiploid (>2 spots) cells: ≤2.5% = ns (not significant); >2.5–5% = ±; 6–10% = +; 11–20% = ++; >20% = +++.
[e]Lymph node metastasis of this tumor revealed the same hybridization pattern.
*$P < 0.01$ (Kolmogorov-Smirnov test).

was seen in the adjacent PIN lesion (Fig. 3E,F). Thirteen PIN lesions (76%) appeared to contain aneuploid cells (mean: 4.5%; Figs. 3G,H, 4). In the adjacent cancer cells a higher degree of aneuploidy was seen (mean: 8.4%). This aberrant ploidy status was not observed in normal cells and BPH (see Materials and Methods).

## DISCUSSION

Over 50% of the examined prostatic cancers showed numerical chromosomal aberrations. Aber-

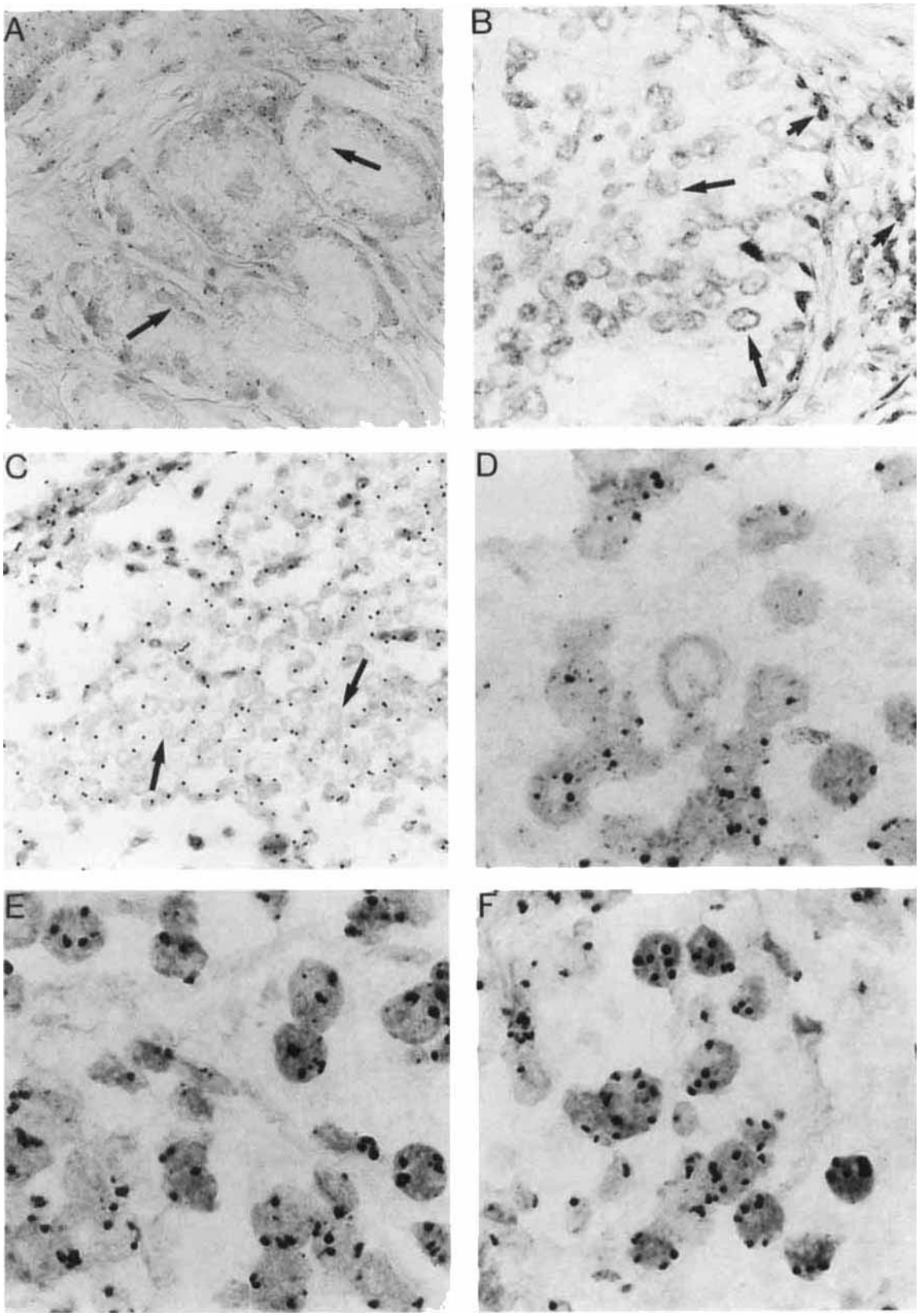



Figure 2. **A:** ISH with the chromosome 8 specific probe to the Gleason 3 area of case 4, showing loss of chromosome 8 in the tumor nuclei (arrows). The ISH-related spots were visualized with immunoperoxidase/DAB (black); hematoxylin was used as a counterstain (gray). **B:** ISH with the chromosome Y specific probe to a Gleason 5 area of case 19 showing a complete loss of the Y chromosome in the tumor cells (large arrows), while the basal and stromal cells carry this chromosome (small arrows). **C:** ISH with the chromosome 15 specific probe to the Gleason 5 area of case 25 showing an underrepresentation of chromosome 15 in the cancer cell nuclei (arrows). The cells display only 1 or 0 spots. **D:** ISH with the chromosome 10 specific probe to the DNA tetraploid Gleason 5 area of case 22. An underrepresentation of chromosome 10 is noted, when compared with chromosome 1 in E. **E:** ISH with the chromosome 1 specific probe to the same area. A large number of aneuploid cells can be distinguished. **F:** ISH with the chromosome 7 specific probe to the Gleason 5 area of the same patient. An overrepresentation of chromosome 7 is seen, when compared with chromosome 1 (E). ×40 (A–C); ×100 (D–F).

TABLE 2. Gleason Score and Chromosomal Aberrations Determined by ISH[a]

| | ≤6 (n = 10) | ≥7 (n = 15) |
|---|---|---|
| +7 | — | 2 (13%) |
| −8/+8 | 1 (10%) | 4 (26%) |
| −10 | 1 (10%) | 2 (13%) |
| −15 | 2 (20%) | 2 (13%) |
| −Y | 2 (20%) | 3 (20%) |

[a]The percentage of tumors within the Gleason subgroups is given in parentheses.

TABLE 3. TNM Staging and Chromosomal Aberrations Determined by ISH[a]

| | T2[b] (n = 5) | T3–T4[c] (n = 20) |
|---|---|---|
| +7 | — | 2 (10%) |
| −8/+8 | — | 5 (25%) |
| −10 | — | 3 (15%) |
| −15 | 2 (40%) | 2 (10%) |
| −Y | 1 (20%) | 4 (20%) |

[a]The percentage of tumors within the TNM subgroups is given in parentheses.
[b]Tumor confined within the prostate.
[c]Tumor invades other organs and/or is fixed.

TABLE 4. Chromosomal Aberrations in Six PIN Compared With Numerical Alterations in Adjacent Adenocarcinoma

| Case | Gleason area | PIN | Adeno-carcinoma |
|---|---|---|---|
| 1 | G2 | — | −Y* |
| 3 | G2 | −Y | −15, −Y |
| 4 | G3 | — | −8 |
| 8 | G3c | — | −15 |
| 9 | G3c | — | −10 |
| 19 | G5 | −Y | −Y |

*$P < 0.01$ (Kolmogorov-Smirnov test).

rations of chromosome 8 and loss of the Y chromosome (both 20%) were the most common findings, followed by loss of chromosomes 15 (16%) and 10 (12%). Gain of chromosome 7 was seen in 8% of cases. No numerical changes of chromosome 1 were observed. Alterations of chromosome 8, loss as well as gain, were seen in five tumors. Classical cytogenetic analyses revealed both monosomy 8 (Brothman et al., 1990; Lundgren et al., 1992a) and trisomy 8 (Micale et al., 1992). Gain of chromosome 8 was also demonstrated by FISH analysis (Macoska et al., 1993; Micale et al., 1993). In RFLP studies loss of alleles from the 8p region was seen in a high percentage of prostatic tumors. Loss of the Y chromosome in prostate cancer has been reported by karyotyping studies. For example, Lundgren et al. (1992a) found loss of the Y chromosome in 40% of the tumors. Loss of chromosomes Y and 10 was found by interphase cytogenetics on cytological material of a metastatic prostate carcinoma (van Dekken et al., 1990a). In our study 16% of all tumors showed loss of chromosome 15. Chromosomal abnormalities of chromosome 15 in prostatic adenocarcinoma have not been reported previously in the cytogenetic literature. Loss of chromosome 10 was seen in 12% of the patients. In the cytogenetic literature a del(10)(q24) has been reported and molecular studies showed allelic loss from the 10p and 10q arms. Monosomy of chromosome 10 was detected by FISH analysis in two tumors (van Dekken et al., 1990a; Micale et al., 1993). Gain of chromosome 7 occurred in 8% of the patients. Gain of chromosome 7 and a del(7)(q22) has been reported in prostate cancer. Recently, FISH analysis suggested that gain of chromosome 7 is associated with the progression of prostate cancer (Bandyk et al., 1994).

In 17 prostatic precancerous lesions adjacent to tumor glands we did not find chromosomal aberrations of chromosomes 7, 8, 10, or 15, even if present in the cancer cells. This illustrates that PIN lesions are distinct entities, which are not created by ingrowth of tumor cells into normal prostatic glands. In two cases we found loss of the Y chromosome in both PIN and adjacent adenocarcinoma. These results suggest that loss of the Y chromosome is an early event in prostatic tumorigenesis. ISH analysis further revealed a high percentage of PIN lesions to be moderately aneuploid. Crissman et al. (1993) found 26% of 87 both low and high grade PIN lesions with coexisting carcinoma to be aneuploid by DNA quantitation. In this study, however, half of the high grade PIN lesions showed aneuploidy.

Almost all chromosomal abnormalities occurred in subsets of tumor cells, irrespective of tumor grade. Genetic heterogeneity within a tumor is presumed to be important in the progression of a tumor to a highly malignant and metastatic state (Lundgren et al., 1992b; Micale et al., 1992). In our study, however, we observed genetic heterogeneity, i.e., subsets of tumor cells carrying a chromosomal abnormality, even in low grade, low stage tumors (Table 1). These cytogenetic growth pat-

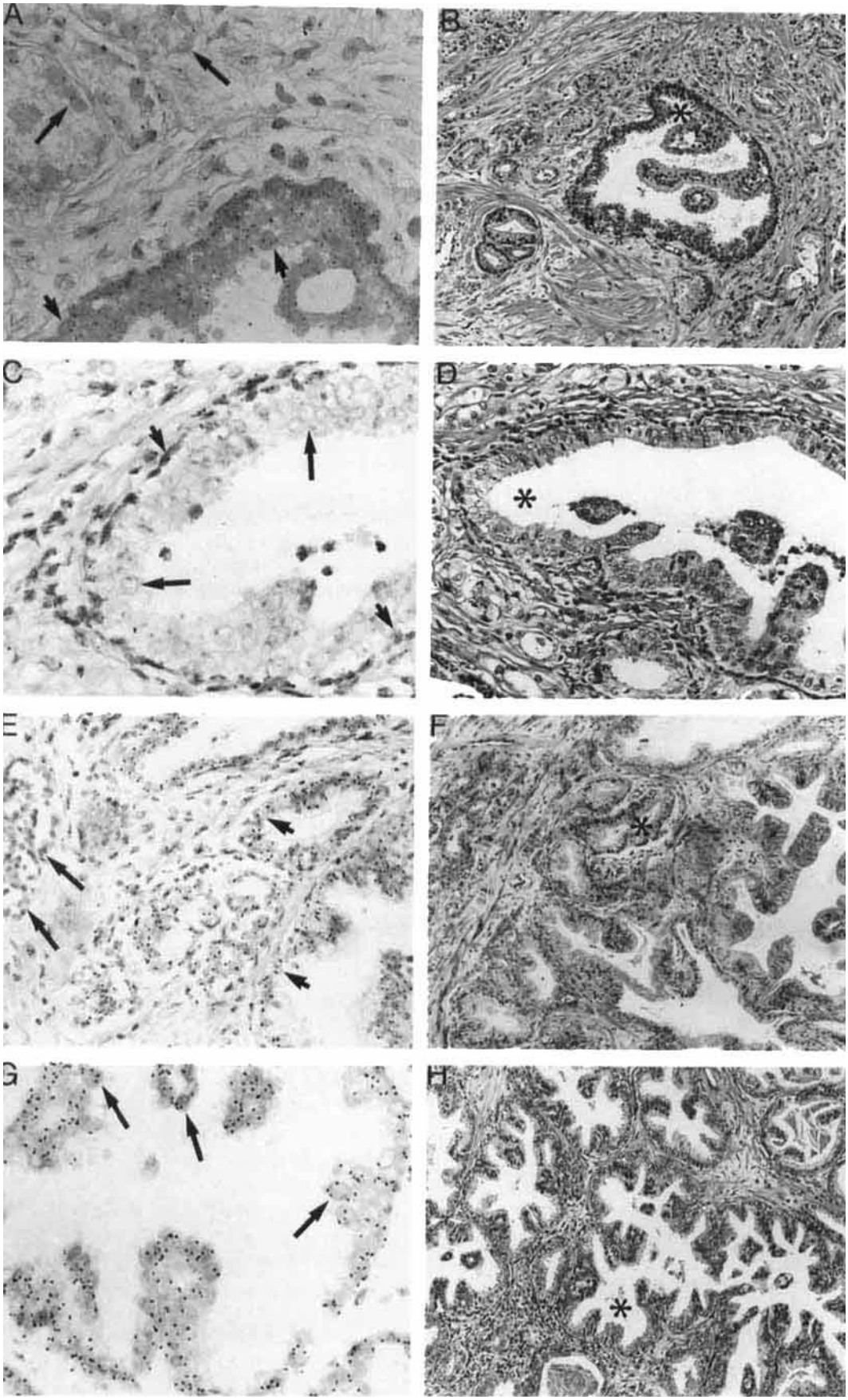



Fig. 3.

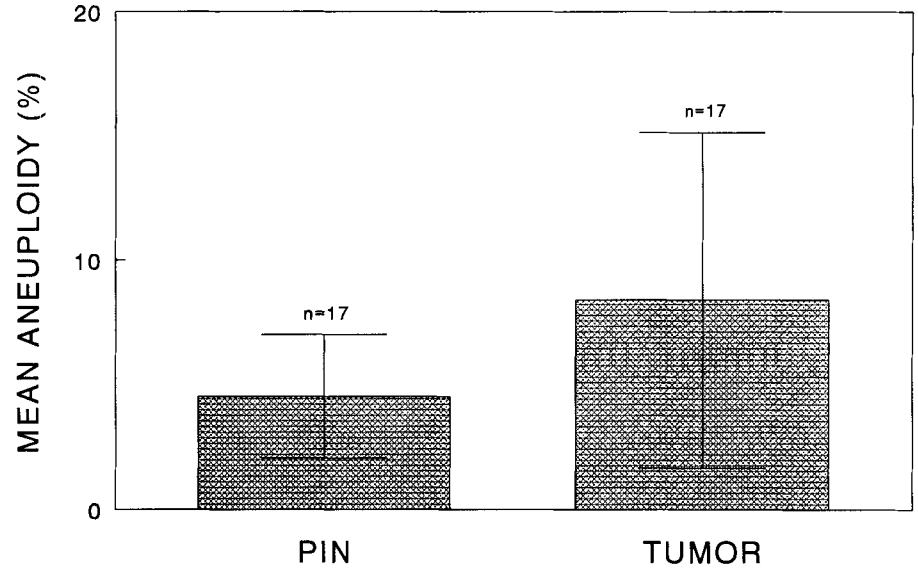



Figure 4. Bar representation of the mean percentages of aneuploidy in prostatic tumors with adjacent PIN. An increase in the percentage of aneuploid cells can be seen in the adenocarcinoma.

terns in prostate cancer will be described in detail in a separate paper (Alers et al., in preparation). Furthermore, in case 19 we observed loss of the Y chromosome in the luminal cells, but not in basal cells in either PIN lesion or tumor cells (Figs. 3C,D, 2B, respectively). The basal cells are considered to contain the stem cells of the prostatic gland (Sell and Pierce, 1994). Thus, if chromosome Y loss is important in prostatic tumorigenesis, our results disagree with the concept of arrest of stem cell differentiation as a leading event in prostatic adenocarcinoma (Sell and Pierce, 1994). We have also seen loss of the Y chromosome in another precancerous lesion, i.e., dysplastic epithelium adjacent to adenocarcinoma of the esophagus (Krishnadath et al., 1994).

In conclusion, interphase ISH to routinely processed paraffin sections of radical prostatectomies revealed genetic abnormalities in all grades and stages of prostatic tumors. It provides a tool to study the cytogenetic events during prostatic tumor progression in which alterations of chromosomes 7, 8, and 10 might be related to more advanced cancers. In the latter tumors high degrees of aneuploidy/tetraploidy were found. ISH to histologic sections allowed us to distinguish aneuploid cells already in the preneoplastic state. Moreover, the occurrence of loss of the Y chromosome in PIN lesions suggests that it is an early event, and a possible biomarker in prostatic tumorigenesis.

Figure 3. **A:** ISH with the centromere 8 specific probe to a PIN within a Gleason 3 area of case 4. Underrepresentation of chromosome 8 is seen in the tumor glands (large arrows), but not in the adjacent PIN lesion (small arrows). **B:** Corresponding hematoxylin and eosin (HE)-stained tissue section. Region of interest is marked by an asterisk. **C:** ISH with the chromosome Y specific probe to a PIN lesion of case 19. Loss of chromosome Y is seen in the luminal cells of the PIN lesion (large arrows), but not in the basal cells (small arrows). **D:** Corresponding HE section of this PIN lesion. Asterisk marks part of this PIN lesion seen in C. **E:** ISH with the Y probe to a PIN lesion adjacent to the Gleason 2 area of case 1. Loss of the Y chromosome is seen in the tumor glands (large arrows), but not in the PIN lesion (small arrows). **F:** Corresponding HE section of the PIN area (asterisk) adjacent to tumor. **G:** ISH with the chromosome 1 specific probe to a PIN lesion of case 12. Several aneuploid nuclei can be distinguished (arrows). **H:** Corresponding HE section of this PIN lesion. Asterisk marks area depicted in G. ×40 (A,C,G); ×20 (D,E); ×10 (B,F,H).

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