

Cytogenetic Heterogeneity and Histologic Tumor Growth Patterns in Prostatic Cancer

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Twenty-five prostatic adenocarcinomas were studied for the presence of intratumoral cytogenetic heterogeneity by interphase in situ hybridization (ISH) to routinely processed tissue sections. ISH with a chromosome Y-specific repetitive DNA probe provided a model to investigate patterns of chromosomal heterogeneity within and between different pathological grades. The Gleason grading system was used, since it is based on a detailed classification of growth patterns. Heterogeneity with respect to ploidy of the tumor was examined by ISH with a repetitive DNA probe specific for chromosome 1. The ploidy status of these cancers was confirmed by DNA flow cytometry ($P < 0.001$). Cytogenetic heterogeneity at the (Y) chromosomal level was observed between Gleason areas, within one area, and even within single tumor glands. The dif-

ferent patterns of chromosomal heterogeneity were seen in all tumor grades and stages. Differences in ploidy status were also found following the aforementioned histological patterns, again, in all grades and stages. Intraglandular heterogeneity was most frequently seen. No correlation was found between cytogenetic heterogeneity and proliferative activity (Ki-67 immunostaining). In contrast to current views on clonality, suggesting regional separation of subclones with different DNA content, this study demonstrates that these subclones can be interspersed. © 1995 Wiley-Liss, Inc.

Key terms: Interphase cytogenetics, in situ hybridization, Ki-67, immunohistochemistry, aneuploidy, multifocality, prostatic adenocarcinoma, archival tissue, chromosomal aberrations

Prostate cancer, which is now exceeding lung cancer as the most commonly diagnosed cancer in American men (9), is known for its highly heterogeneous histological appearance (13). Foci within a prostate show varying degrees of differentiation and may contain cells that differ genetically. Additionally, the complex tissue architecture [often comprising normal epithelium, stromal cells, benign prostatic hyperplasia (BPH), and prostatic intraepithelial neoplasia (PIN) within the tumor mass] has complicated analysis of prostatic tumors by conventional cytogenetic analysis. Karyotyping studies of prostate cancers are further hampered by the low-mitotic index of prostatic tumor cells and subsequent overgrowth in tissue culture of (normal) stromal or epithelial cells. Karyotyping of prostatic tumors has shown recurrent chromosomal aberrations of chromosome arms 7q and 10q, loss of Y, and trisomy of chromosome 7 (2,5,27,29), and restriction fragment length polymorphism (RFLP) studies have revealed loss of alleles on 8p, 10p, 10q, 16q, and 18q arms (3,6,25). However, a clinically important chromosomal abnormality in prostatic cancer has yet to be identified.

In general, cytogenetic heterogeneity is a common feature of solid (epithelial) tumors (17,21,28,32,43). Only limited data are available concerning cytogenetic heterogeneity in prostatic tumors (4,26,29). Lundgren et al.

(26) have demonstrated by karyotyping studies that patients with clonal chromosomal abnormalities had a poor outcome, compared with those who had non-clonal aberrations. Intratumoral heterogeneity in ploidy status of prostatic tumors has also been revealed by DNA flow cytometry (FCM) (4,24,31). Both aneuploidy/tetraploidy and diploidy have been detected when several biopsies per tumor were analyzed (4,24,31). Likewise, FCM DNA studies of multiple samples from different sites in one tumor and/or metastases have shown heterogeneity in DNA in lung cancers (7,38), gliomas (10), pancreatic tumors (39), gastrointestinal cancers (11,38), and ovarian carcinomas (15). In addition, in epithelial tumors such as bladder cancer (19,20,35), breast cancer (12), and lung cancer (22), cytogenetic heterogeneity has been revealed by interphase cytogenetics.

Interphase cytogenetic analysis by in situ hybridization (ISH) has been increasingly utilized to detect specific

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chromosomal abnormalities and their relation to progression in neoplasms. Most investigators have used cell suspensions from disaggregated tumor blocks (e.g., 19,41). However, the inevitable loss of tissue architecture prevents the detection of relationships between chromosome changes and histopathological characteristics. To circumvent these problems, we have applied ISH to archival paraffin-embedded tissue sections (23,40,42).

In the present study we addressed the following questions: 1) Does chromosomal heterogeneity exist within prostatic tumors, and if so, at what histological levels (glands, areas)? 2) Is the ploidy status of the tumors heterogeneous? and 3) Are there any correlations between cytogenetic characteristics and proliferative activity of the tumor cells?

MATERIALS AND METHODS

Tissue Preparation

Routinely processed, formalin-fixed, paraffin-embedded tissues, 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 (36) and graded according to the Gleason system (13). The Gleason grading system recognizes five growth patterns with increasing loss of histological differentiation from grade 1 to 5. A tumor is assigned a certain Gleason score by adding the grades of the two dominant growth patterns (13). Forty-one Gleason areas were discriminated in our panel of 25 prostatic tumors.

In situ hybridization (ISH) was performed on consecutive 4 μ m tissue sections. Sections were mounted with distilled water on aminoalkylsilane (Sigma, St. Louis, MO) coated microscope glass slides and baked overnight at 60°C for better adherence.

Probe Selection

Probes specific for chromosome 1 and Y were selected. Selection criteria were based on literature data considering numerical (and structural) aberrations in prostate and other solid tumors. As described above, loss of the Y chromosome is reported in prostatic cancer (2,5,27, 29,40). Furthermore, the Y probe was best suited for visualizing heterogeneity in the prostatic tumors, since loss of the Y chromosome is easily recognized. A probe specific for the centromeric region of chromosome 1 was chosen to quantify the rate of aneuploidy of the tumors. No recurrent numerical aberrations were found for this chromosome in karyotyping studies (2,5,27,29), as well as in an ongoing investigation by our own group (1).

In Situ Hybridization

ISH was performed as previously described (23,42). The (peri)centromeric repetitive satellite DNA probes were labeled with biotin-14-DATP by nick translation of complete plasmid DNA according to the manufacturer's directions (BRL, Gaithersburg, MD). Briefly, tissue sections were deparaffinized and then treated with 0.3% H₂O₂ in methanol for 20 min to block endogenous per-

oxidase activity. To facilitate DNA probe accessibility to the cellular DNA, sections were digested with 0.4% pepsin (Sigma) in 0.2 M HCl at 37°C for 5–30 min (mean, 14 min), after an incubation in 2 \times standard saline citrate (SSC; pH 7.0) at 70°C for 30 min to shorten the digestion time.

Both cellular DNA and the chromosome-specific repetitive DNA probes were heat denatured. The hybridization mixture contained 1–2 μ g/ml probe DNA, 500 μ g/ml sonicated herring sperm DNA (Sigma), 0.1% Tween-20, 10% dextran sulphate, and 60% formamide in 2 \times SSC at pH 7.0. The slides were then incubated overnight at 37°C and subsequently washed.

Histochemical detection was performed by immunoperoxidase diaminobenzidine (DAB) staining as previously described. Finally, the sections were counterstained with hematoxylin, rinsed in tap water, dehydrated, and mounted in Malinol (Chroma-Gesellschaft, Köngen, Germany).

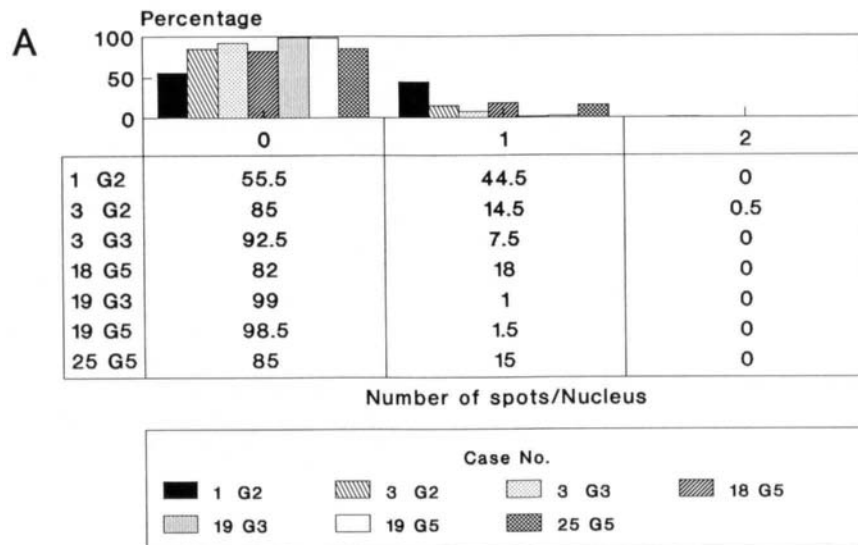
Evaluation of ISH results

The centromere 1 and Y DNA probes, as well as the autosomal control DNA probes, were analyzed for each prostate adenocarcinoma on consecutive 4 μ m sections in a tumor area to which a certain Gleason score had been assigned. A section size of 4 μ m was chosen after evaluating the degree of nuclear overlap (i.e., countability) and section thickness. On each tissue section leukocytes, BPH, nerve cells, etc., served as internal controls to evaluate the quality of ISH and to detect probe polymorphisms. For each of the probes, 100 "intact" (i.e., spherical) and non-overlapping 4 μ m nuclear slices were counted by two independent investigators and the number of solid DAB spots per nuclear contour was scored (0, 1, 2, 3, 4, >4 spots/nuclear slice). The individual DNA probe spot distributions were then compared and tallied, when no significant counting differences between the investigators were found. In our series no discrepancies emerged using this approach. Tumor aneuploidy was determined by calculating the percentage of hyperdiploid cells in the dominant Gleason area(s). Heterogeneity for chromosomes 1 and Y was scored by both careful inspection and counting of the aberrant areas.

Validation of ISH results

Two types of artefacts could interfere with the analysis of heterogeneity, defined by ISH to routine tissue sections: 1) the effect of truncation of the nuclei, which causes disturbances, (most importantly) at the tumor glandular level; and 2) the efficiency of hybridization, leading to regional differences. Loss of the Y chromosome within one tumor gland was not caused by artefacts due to truncation of nuclei. Previous studies by our group (23,42) revealed that an average of 65% of the cells displays an ISH Y-spot in truncated (normal) nuclei of various histologies. This distribution is in sharp contrast to tumor glands with loss of Y. Furthermore, to rule out a possible contamination of Y-less tumor cells within

TUMORS



CONTROLS

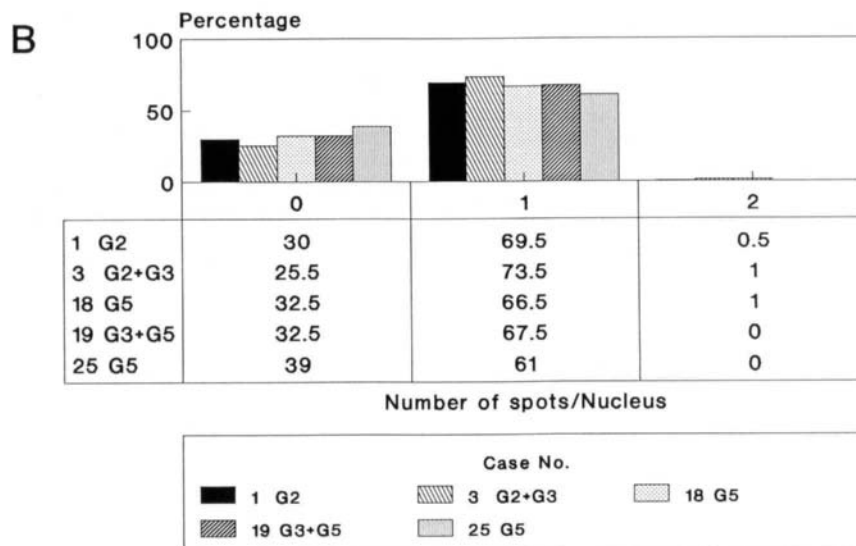


FIG. 1. Bar histograms showing ISH spot distributions for the chromosome Y-specific DNA probe on 4 μ m tissue sections. **A:** ISH patterns of Gleason areas with (partial) loss of Y in cases 1, 3, 18, 19, and 25. **B:** Control cells from the same sections of the aforementioned cases (e.g., leukocytes, normal prostatic glands). In control cells the percentage of cells with 1 spot for Y is between 60 and 70%. Note that Y-loss is seen as a shift to the left in the distributions.

one gland with normal pre-existent cells that still carry the Y chromosome, cytopathology was checked in adjacent hematoxylin and eosin-stained slides. In case of intraglandular heterogeneity for ploidy, truncation of the nuclei cannot result in spot distributions as observed in this study. If a tumor gland were to be fully tetraploid or

aneuploid, a distribution of ISH spots would be created with more than 50% of the cell nuclei carrying three or four hybridization spots for chromosome 1, as described previously by us (42). By contrast, we most frequently observed a few tetraploid cells in a diploid background.

Insufficient ISH, leading to regional artefacts, can be

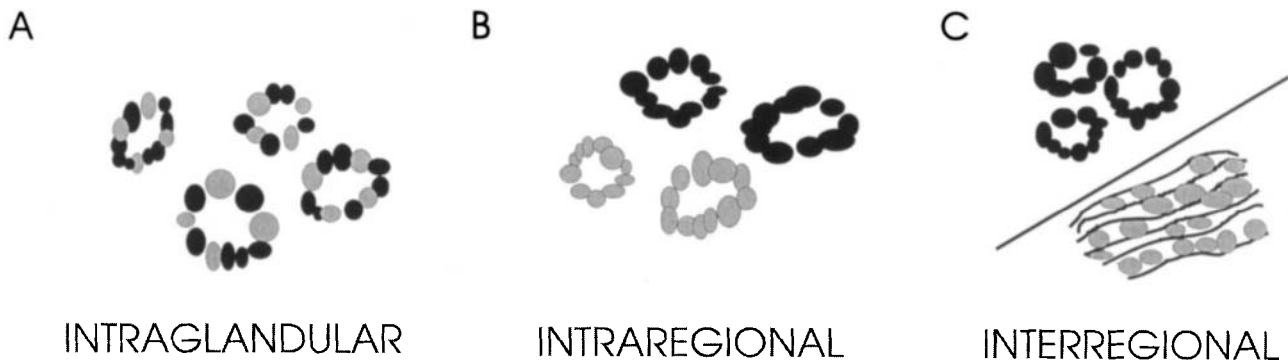


Fig. 2. Schematic drawing of histological patterns of cytogenetic heterogeneity in prostatic adenocarcinoma as detected by interphase cytogenetics. Nuclei that are different with respect to chromosomal and/or ploidy status are depicted in different shades. Three distinct histological patterns were discriminated. **A:** Intraglandular heterogeneity: neighbor-

ing cells are cytogenetically different. **B:** Intraregional heterogeneity: within one Gleason area, neighboring tumor glands show cytogenetical differences. **C:** Interregional heterogeneity. One Gleason area is cytogenetically different from another.

ruled out for the following reasons: 1) stromal cells adjacent to tumor glands always showed strong ISH signals, thus eliminating focal misinterpretations; and 2) ISH was controlled in adjacent sections hybridized with autosomal DNA probes; this prevented false interpretations at a more regional level. We never observed areas within one section with loss of ISH signal in both tumor and neighboring stromal cells. These artefacts could be seen when the tissue had different accessibility for the probe in different areas (e.g., due to variable fixation).

Immunohistochemistry

Primary labelling of the Ki-67 antigen was performed with a monoclonal antibody, MIB-1 (Immunotech, Marseille, France), diluted 1/100 in phosphate-buffered saline (PBS) (8). Immunohistochemistry was performed using the routine ABC-immunoperoxidase method (Vector). For each specimen 200 tumor cells in the marked Gleason areas were counted randomly by two independent investigators.

DNA Flow Cytometry

DNA content of the paraffin material was measured as described by Hedley et al. (16). Gleason-graded tumor areas were selectively cut out of the paraffin blocks. In eight cases (32%) the two dominant Gleason growth patterns were present within the same tissue block and could not be separated. Correspondence (presence of tumor, tumor grade) between upper and lower boundary was seen in 93% of the tumor samples. In only 3 of 41 areas (7%) was the tumor area not present at the lower boundary. Flow cytometry 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 0.9 and 1.1 was considered diploid. The percentage of non-2C peak cells

was derived from the flow histograms and used to determine the percentage of aneuploid/tetraploid cells of the dominant Gleason area(s) within one tumor.

RESULTS

Chromosomal Heterogeneity

Loss of chromosome Y was used as a model system to study chromosomal heterogeneity. In our panel of 25 radical prostatectomies we found loss of the Y chromosome in five cases (seven Gleason-graded areas; Fig. 1A). Loss of the Y chromosome was never observed in control cells, present on the same tissue section (Fig. 1B). Loss of Y was seen in both low and high grade tumors (Table 1). Heterogeneous loss of this chromosome was seen at three levels of aggregation: intraglandular, intraregional,

Fig. 3. (Figure on page 88.) **A:** ISH with the chromosome Y-specific DNA probe to the Gleason 2 area of case 3, showing intraglandular chromosomal heterogeneity: loss of chromosome Y can be seen in most tumor cell nuclei (large arrows), whereas some cancer cells still contain the Y chromosome (small arrows). At least ten neighboring cancer cell nuclei with Y-loss are seen to line up in this gland. The ISH-related spots were visualized with immunoperoxidase/DAB (black), and hematoxylin was used as a counterstain (gray). **B:** ISH with the Y-specific probe to the Gleason 2 area of case 1, showing intraregional heterogeneity. Foci of tumor glands without Y chromosome (large arrows) and with chromosome Y (small arrows), both situated in one Gleason area, are shown. **C:** Detail of B, showing Y-loss in tumor glands. **D:** Detail of B, showing retention of Y in other tumor glands. **E:** ISH with the chromosome 1-specific (control) probe to the same area of case 1. No differences in ISH-related spot pattern for this probe can be seen between foci without (large arrows) and with (small arrows) the Y chromosome, thus eliminating the possibility of different hybridization efficiency between the two areas. **F:** ISH with the Y probe to the Gleason 5 area of case 18. Y-loss is seen in the cancer cells (large arrows), but not in the stromal cells (small arrows). **G:** Same case, showing the Gleason 3 area: in these tumor glands the Y chromosome is present. This case illustrates interregional chromosomal heterogeneity. A 40 \times objective was used in A, C, D, F, and G and a 20 \times objective in B and E.

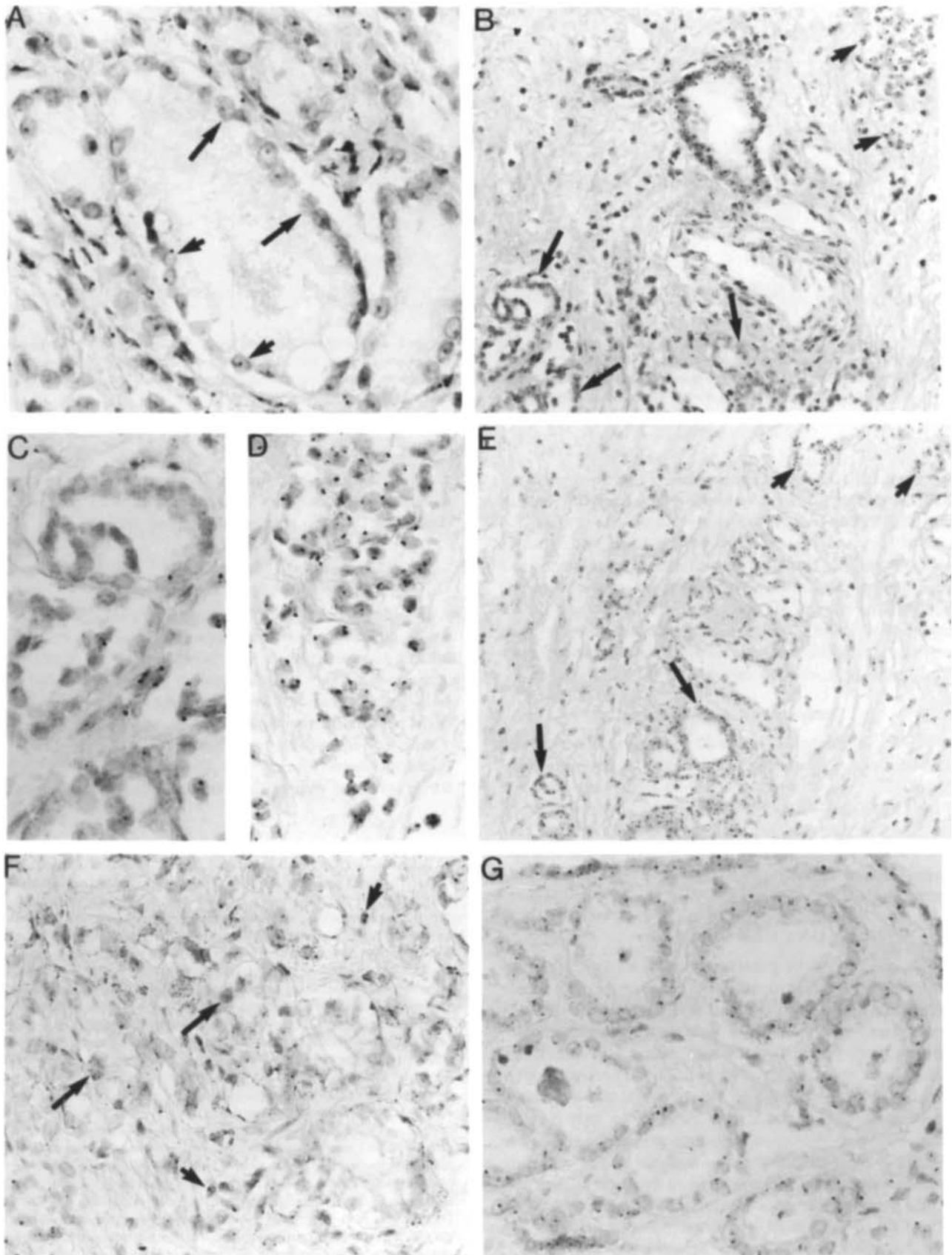


FIG. 3.

Table 1
Results of Pathological Grading/Staging, Flow Cytometry (FCM), and In Situ Hybridization (ISH)

Case	Grade	Type ^a	Stage ^b	FCM ^c	ISH aneuploidy ^d (%)	Y-ISH
1	4	G2	T3N0	D (7)	4	-Y
2	5	G2	T4N2	D (8)	6	
		G3			2.5	
3	5	G2	T2N0	D (8)	23	-Y
		G3			21.5	-Y
4	5	G2	T3N0	D (5)	8	
		G3		A ^c	6.5	
5	5	G2	T2N0	T (19)	37.5	
		G3			3	
6	5	G2	T2N0	D (7)	0.5	
		G3		T (12)	1	
7	6	G3c	T2N0	T (14)	8	
8	6	G3c	T3N0	D (9)	1.5	
9	6	G3c	T3N0	D (10)	4.5	
10	6	G3	T3N0	D (7)	1	
11	7	G3	T3N0	D (6)	2	
		G4		T (21)	5.5	
12	7	G3	T3N0	T (23)	2	
		G4			9.5	
13	7	G3c	T3N0	A (20)	12	
		G4		T (36)	30.5	
14	7	G3	T3N0	D (10)	1	
		G4		D (8)	2	
15	7	G3	T3N0	D (4)	2	
		G4			24.5	
16	7	G3c	T4N0	T (16)	46	
		G4			4.5	
17	8	G3	T3N0	A ^c	32	
		G5		T (36)	21.5	
18	8	G3	T4N0	D (1)	1.5	
		G5		D (4)	2	-Y
19	8	G3c	T3N0	A (23)	19	-Y
		G5			18	-Y
20	8	G4	T3N1	T (19)	23	
21	9	G4	T3N0	D (9)	4	
		G5			2.5	
22	9	G4	T4N0	D (7)	30	
		G5		T (44)	41	
23	10	G5	T2N0	T (40)	42	
24	10	G5	T3N0	D (5)	15.5	
25	10	G5	T3N0	T (22)	6.5	-Y

^aDominant Gleason growth pattern(s).

^bTNM classification: All tumors M0.

^cD(iploid): $0.9 < Di < 1.1$ and 4C peak $\leq 10\%$; T(etraploid): $0.9 < Di < 1.1$ and 4C peak $> 10\%$; A(neuploid): $Di < 0.9$ or $Di > 1.1$. Values between parentheses are percentage non-2C peak FCM.

^dPercentage of cells with > 2 spots/nuclei for chromosome 1. All control cells revealed $< 2.5\%$ hyperdiploid cells.

^eShoulder in the DNA histogram.

and interregional (Fig. 2). In detail, loss of the Y chromosome was seen in some cells within one tumor gland, whereas other cells in the same gland still contained the Y chromosome, thus displaying intraglandular heterogeneity (Figs. 2A, 3A). Foci of glands that lost chromosome Y and foci of glands that showed the normal spot distribution for Y alternated within one Gleason area (intra-regional heterogeneity; Figs. 2B, 3B–E). The third distinct pattern of heterogeneity, termed interregional heterogeneity, defined loss of the Y chromosome in one Gleason area, whereas the other Gleason area retained the chromosome (Figs. 2C, 3F,G; Table 1). Furthermore, the three different patterns of chromosomal heterogeneity

FIG. 4. A: ISH with the chromosome 1-specific probe to the Gleason 2 area of case 3. Intraglandular differences in ploidy can be seen between neighboring cells. A few aneuploid cell nuclei with three or four spots are arrowed. B: Same case: intraglandular heterogeneity showing slightly more aneuploid nuclei (arrows). Note that, in general, these nuclei are larger in size than those with one or two spots. C: Same case: ISH with the chromosome 7-specific (control) probe to the same area. This probe displays the same ploidy pattern. A few aneuploid nuclei are arrowed. D: ISH with the centromere 1-specific probe to the Gleason 2 area of case 5. Many aneuploid nuclei can be distinguished, and a few are arrowed. E: Neighboring cancer glands within the same area contain cells mostly showing two spots per nucleus (intra-regional heterogeneity). F: Gleason 3 area: ISH with the centromere 1 probe to the cribriform gland (case 16). Many aneuploid nuclei are visible. G: Same case, Gleason 4 area: most nuclei display two spots. Case 16 illustrates interregional heterogeneity in ploidy. A 40 \times objective was used in A–G.

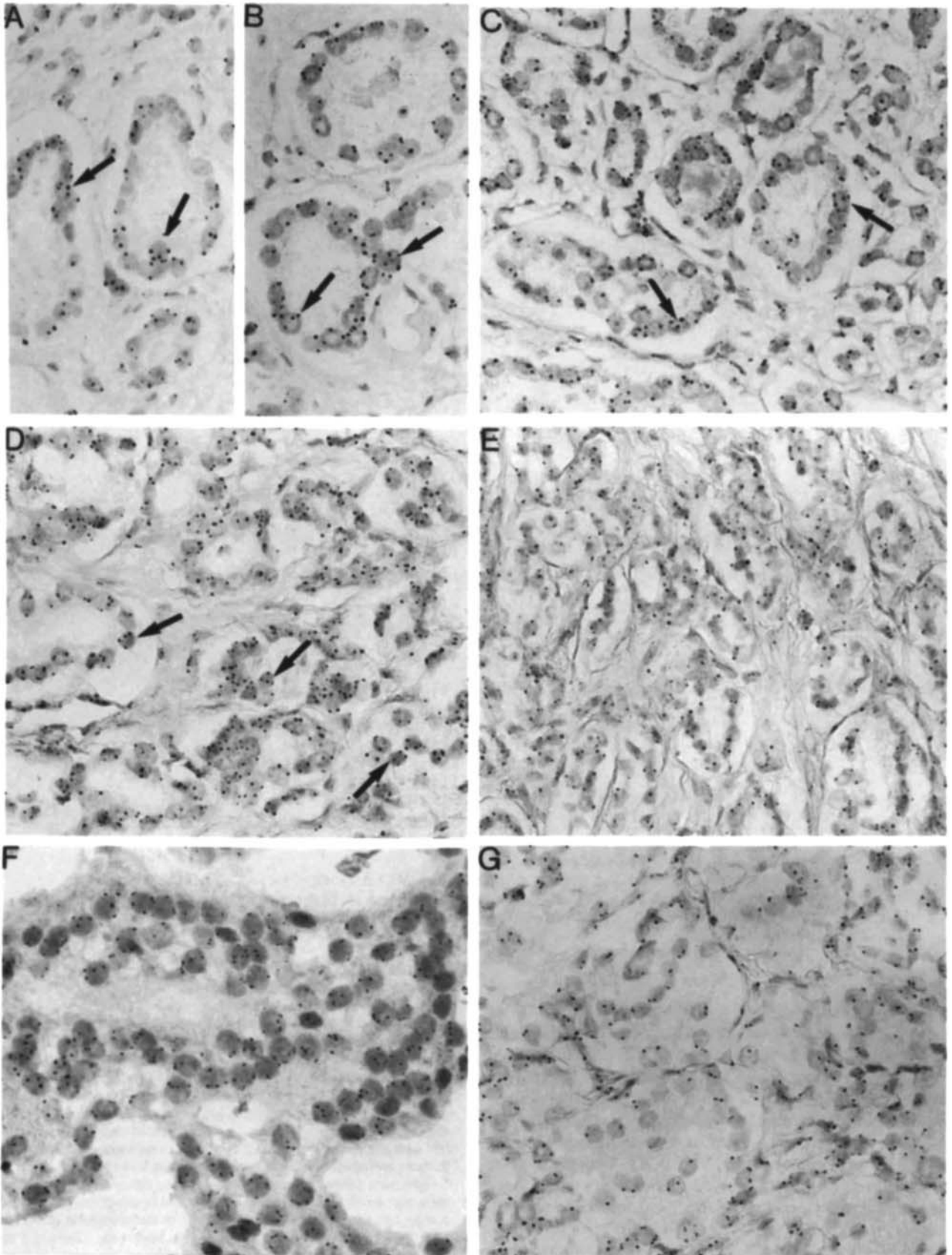
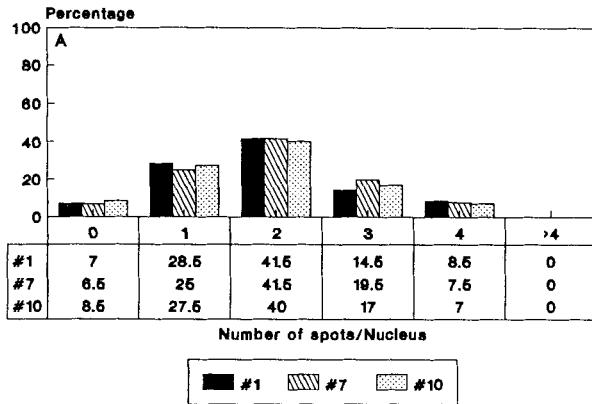
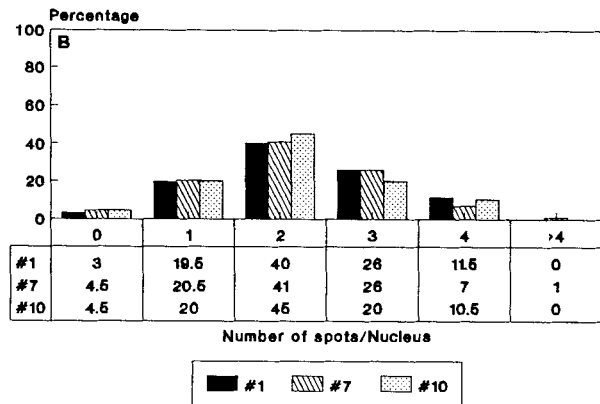


FIG. 4.

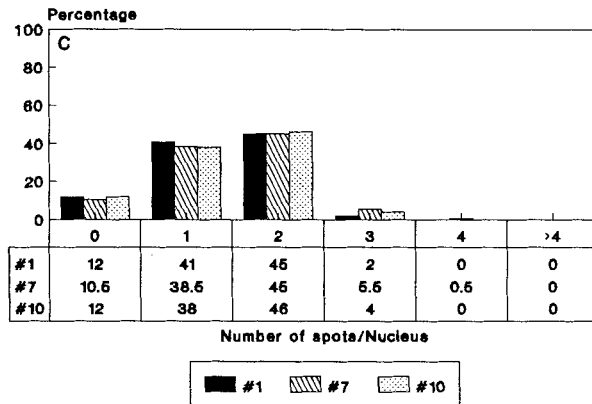
CASE 3 (G2)



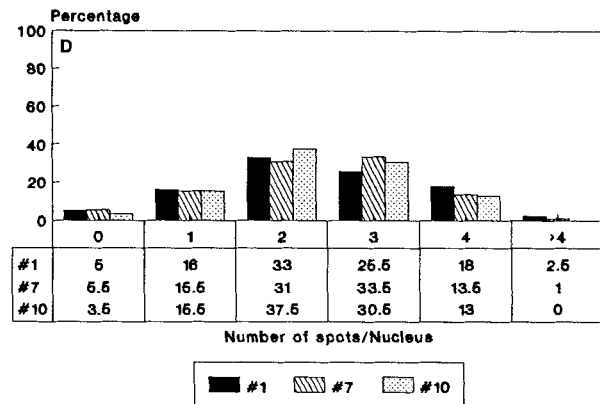
CASE 5 (G2:Area 1)



CASE 5 (G2:Area 2)



CASE 16 (G3)



CASE 16 (G4)

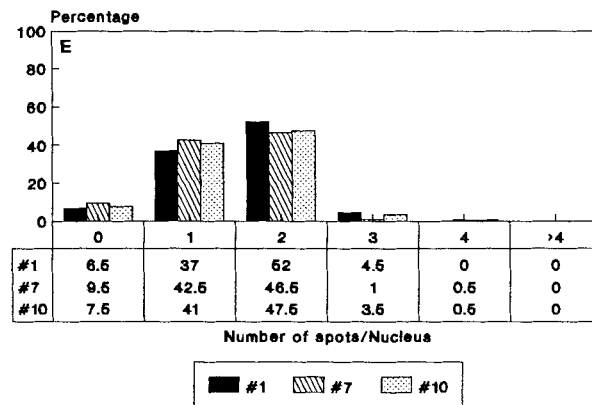


Fig. 5. ISH spot distributions for chromosomes 1, 7, and 10 of consecutive tissue sections of all cases shown in Figure 4. A: Gleason 2 area of case 3. B: Aneuploid part of Gleason 2 area of case 5. C: Less aneuploid part of Gleason 2 area of case 5. D: Gleason 3 area of case 16. E: Gleason 4 area of the same case. The spot distributions for these chromosomes are highly comparable. It illustrates equal hybridization conditions for all these probes.

ity, illustrated by Y-loss, occurred in both low-grade ($n = 2$; Gleason score ≤ 6) and high-grade ($n = 3$; Gleason score ≥ 7) tumors.

Heterogeneity in Ploidy

A probe specific for the centromere region of chromosome 1 was used to assess the ploidy status of the tumor cells within the dominant Gleason growth pattern(s) (Table 1). Aneuploidy defined by ISH correlated well with aneuploidy/tetraploidy measured by DNA FCM ($P < 0.001$, Pearson's rank correlation). Heterogeneity in ploidy, demonstrated by ISH with the DNA probe specific for chromosome 1, revealed the same three patterns that were distinguished for chromosomal heterogeneity (Fig. 2). Intraglandular heterogeneity resulted in differences in spot number for chromosome 1 between neighboring nuclei (Figs. 2A, 4A,B). Control probes for other chromosomes, e.g., chromosome 7, showed comparable hybridization patterns in the same tumor glands (Figs. 4C, 5A). Intra-regional differences within one Gleason area were seen in, for example, the Gleason 2 area of case 5. One part of this area was highly aneuploid, whereas another part showed a more diploid distribution (Figs. 4D,E, 5B,C). Interregional heterogeneity for ploidy between different Gleason areas was distinguished in several cases (Table 1). This is illustrated by an aneuploid cribriform growth pattern (grade 3) and another poorly differentiated area (grade 4) of case 16, which displayed a rather diploid distribution (Figs. 4F,G, 5D,E). In all these cases control probes showed the same spot distribution as chromosome 1 (Fig. 5). No significant differences in the occurrence of the three aforementioned histological patterns were observed between low- and high-grade tumors (Fig. 6). Intraglandular heterogeneity was most frequently detected.

Proliferative Activity

Immunohistochemistry (IHC) with a Ki-67 antibody (MIB-1) in normal and hyperplastic glands demonstrated less than 2.5% positive immunostaining of the nuclei. In tumor cells the percentages of stained nuclei varied from 1% to 28% (mean, 8%). No differences of MIB-1 staining patterns were observed between parts of a tumor with or without chromosome (Y) loss (not shown). Likewise, heterogeneity in ploidy of the tumor cell nuclei did not result in differences in proliferation rate as assessed by MIB-1 IHC.

DISCUSSION

In this study we were able to distinguish cytogenetic heterogeneity in prostatic adenocarcinomas by means of ISH to routine paraffin sections. This approach retained the tissue architecture, allowing detection of cell subsets with different karyotypes. Control studies were performed for, e.g., the effect of truncation of the nuclei and variation in hybridization efficiency. Three patterns of cytogenetic heterogeneity could be distinguished: intraglandular, intraregional, and interregional (Gleason areas). Heterogeneity for both chromosomal status and

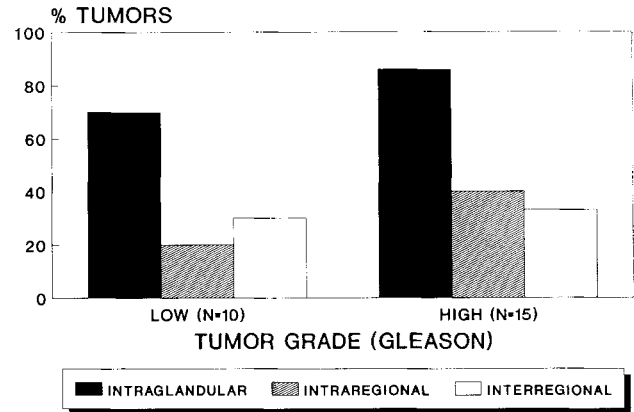


Fig. 6. Bar histogram of the distribution of the three different patterns of heterogeneity in ploidy with respect to pathologic grade. Heterogeneity of ploidy status can be found in both low- and high-grade tumors.

chromosome 1 ISH defined ploidy occurred in these three patterns. Cytogenetic heterogeneity at the chromosomal level was defined by loss of chromosome Y. Loss of Y is the most common chromosomal aberration in prostatic cancer (2,5,27,29). The importance of (loss of) the Y chromosome is not clear. In some cancers loss of Y is a possible prognostic parameter (33). In our panel no correlation was found between age of the patient and loss of the Y chromosome, as has been reported previously (34). Further, control cells, e.g., leukocytes, always retained the Y-chromosome. We observed heterogeneity for Y-loss even within one tumor gland. In our series of tumors we did not observe significant differences in the occurrence of the described histological patterns for Y-loss heterogeneity between low- and high-grade tumors. Although the number of tumors with Y-loss is too small for statistical evaluation, our data suggest that with tumor progression the Y-loss pattern does not change dramatically.

Chromosome 1 was used as a measure for ploidy status. In a previous study no abnormalities of this chromosome occurred (1). Further, tumor aneuploidy, defined by ISH for chromosome 1, was confirmed by DNA FCM. Similar results were obtained by us in other organ systems (23,42). FCM is not suitable for the detection of subtle focal differences. This study shows that neighboring nuclei within one tumor gland contained different copy numbers of chromosome 1 and therefore they differ in DNA content. Surprisingly, it appeared to be the most frequent ploidy pattern. These data suggest that "single cell heterogeneity" exists aside from focal differences. The latter is seen by us as intra- and interregional heterogeneity. In our study we did not observe significant variation in the incidence of the three types of heterogeneity between low- and high-grade tumors. Apparently, in high-grade tumors ploidy differences can still exist at various levels, due to continuous genetic instability, rather than expansion into one single pattern (30).

Clonal karyotypic changes in prostatic tumors were also found by others (4,26,29). In most karyotyping stud-

ies a clone is defined as two or more cells with the same karyotype, or three or more cells with the same numerical aberration. Micale et al. (29) reported that clonal aberrations were confined to tumors in advanced stages. Henke et al. (18) used interphase cytogenetics and found that focal abnormalities occurred only in higher tumor grades. These findings are in contrast to our data: We distinguished cytogenetic heterogeneity throughout the grading and staging spectrum. DNA FCM of multiple samples of prostatic adenocarcinomas showed heterogeneity in ploidy in more than half of cases (31). This multiple site sampling demonstrated that single biopsy specimens, when used in karyotyping, DNA FCM, and interphase cytogenetics on nuclear suspensions, are hardly representative for a given (prostatic) tumor (4,7,31,37,38). At present, the clinical importance of DNA ploidy heterogeneity is not clear and varies among the tumours studied (10,14,15).

We conclude that cytogenetic heterogeneity is a very prominent feature of prostatic adenocarcinoma. ISH applied to routine sections provides us with a tool to discriminate this phenomenon even at the glandular level.

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