Genotypic Characterization of Prostatic Carcinomas: A Combined Cytogenetic, Flow Cytometry, and in Situ DNA Hybridization Study

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ABSTRACT

Cytogenetic studies were performed on 36 biopsies obtained from 26 primary prostatic adenocarcinomas. Following histopathological characterization of control sections, the biopsies were investigated using metaphase cytogenetics, DNA flow cytometry, and fluorescence in situ DNA hybridization. In 12 specimens, no carcinoma was found in control sections by histopathological means. In 24 carcinoma biopsies clonal aberrations were detected in 15 specimens. Tetraploidy as sole aberration was detected in five specimens. Loss of the Y chromosome was seen in eight samples. Only one tumor revealed structural abnormalities. Eight samples were found to be normal (46,XY). Remarkably, nonclonal chromosomal aberrations, particularly marked chromosome loss, were frequently detected in prostatic carcinomas and premalignant lesions (prostatic intraepithelial neoplasia). In the series of biopsies investigated by means of cytogenetics and flow cytometry, biopsies with aneuploid DNA content were found to be cytogenetically normal. Conversely, the cytogenetically aberrant clones were found to be of diploid DNA content. Evidence of focal intratumoral heterogeneity was revealed by cytogenetics, flow cytometry, and in situ hybridization.

INTRODUCTION

In the last decades, prostate cancer has become the most common cancer in males aged 60 yr and over in Western countries and still increases in incidence (1, 2). Despite the knowledge of prognostic factors (tumor grade and stage, performance status, prostate-specific antigen, prostatic acid phosphatase, androgen receptor status, DNA ploidy), the individual outcome of the disease is not yet predictable because of a wide interindividual variety of progression behavior (3). A poorly understood, unique feature of prostate carcinoma is the high frequency of normal karyotypes found in prostatic carcinomas and to refine the approach to intratumoral focal heterogeneity.

MATERIALS AND METHODS

Tissue samples from patients with prostatic adenocarcinoma were examined after radical prostatectomy. Two to three biopsies of approximately 1 cm² were taken from different areas of each cancerous prostate, macroscopically supposed to be situated within the tumor. Each biopsy was subdivided to allow parallel characterization by different techniques and was processed separately.

Cell Culturing and Immunophenotyping. The tissue was disaggregated mechanically and/or enzymatically. Cell culture was processed with a minimal essential medium/F-12 medium supplemented with 10% fetal calf serum, antibiotics (penicillin/streptomycin), and growth factors (epidermal growth factor, cholera toxin, insulin, hydrocortisone) as described earlier (14). This culture system is supposed to select against fibroblast growth. The epithelial nature of the outgrown cells was verified routinely by evaluation of morphology and by immunocytochemical staining for keratins 8, 18, and KL-1.

Cytogenetics. Cytogenetic studies were performed on 36 biopsies obtained from 26 primary, not pretreated prostatic adenocarcinomas and on 2 samples from metastatic iliac lymph nodes. For control, 5 prostatic tissue samples from 2 young men without any prostatic disease were analyzed.

Cytogenetic preparation followed standard protocols. Evaluation of at least 15 metaphases was sought from each sample. In 5 samples with extremely low mitotic activity in vitro, only 7 to 12 metaphases were evaluable (see Tables 1 to 3). Chromosomes were classified and listed according to the International System for Cytogenetic Nomenclature (ISCN) (15). A clone was defined as at least two cells with the same structurally rearranged or additional chromosome or three or more cells with the same missing chromosome.

Flow Cytometric DNA Analysis. Portions of 23 fresh biopsies were analyzed, 11 of them complementary to successful cytogenetic study. Nuclear suspensions were prepared according to the procedure described by Otto (16). The fluorescence of the DAPI-stained nuclei was analyzed in a Partec PAS III flow cytometer (Partec AG, Münster, Germany) and displayed as histograms of DNA content versus number of nuclei. Each distinct peak in the DNA histogram was considered to represent a cell population. A standard sample of human male lymphocytes was admixed to aliquots of tumor sample (internal standard). Approximately 10,000 to 15,000 cells from each sample were analyzed. A DNA diploid sample was defined as displaying a histogram with only one symmetrical G0-G1 peak placed at the diploid level and was assigned a DI of 1.0 (17). DNA aneuploidy was defined by one or more additional peaks, distinguished from the G0-G1 and G2-M peaks. The DI of an aneuploid peak was calculated by dividing the modal channel number of the peak with the modal channel number for the diploid cells.

FISH. Five- to 8-µm sections were made from paraffin-embedded tissue of 12 biopsies. For precise histomorphological correlation of FISH-detected abnormalities, sections for FISH were alternated with those for histological staining. A chromosome 1 (peri-)centromeric probe (pUC1.77) (18) and a Y q heterochromatin-specific probe (pY3.4) (19) were used simultaneously in a two-color fluorescence in situ hybridization.
detected using the applied protocol was designed particularly for paraffin-embedded tissue sections of prostatic carcinomas by Lutz et al. (20).

Analysis of the signal pattern included search for focal losses of the Y chromosome. As a screening for clonal and heterogeneous aneuploidy of chromosomes 1 and Y, two major disadvantages of the FISH tissue section technique applied on prostatic carcinoma had to be overcome: most nuclei in the sections were found to be cut off, and solid carcinoma areas showed overlapping nuclei. Therefore, exact analysis of the chromosomal composition of individual nuclei was not possible. For this purpose, the following evaluation protocol was used. In 5 different areas per slide, 100 Y-specific signals were correlated with the corresponding number of chromosome 1-specific signals. A normal quotient serving as standard was determined using normal prostatic epithelium. In one case with a conspicuous quotient, FISH was performed on isolated nuclei of the same biopsy.

Histopathological Examination. In addition to routinely performed histopathological examination of the surgically removed prostate, each biopsy, which was included in our study, was separately analyzed following standard staining protocols. The Tumors/Nodes/Metastases (TNM) system (21) was used for tumor staging and the WHO-recommended grading system (22), for grading.

RESULTS

Cytogenetic Studies. Thirty-six biopsies taken from cancerous prostates were examined. In 12 specimens, histological analysis of control sections adjacent to the tissue submitted for culture revealed lack of carcinoma cells.

A compilation of cytogenetic data from the 24 carcinomatous specimens is shown in Table 1. Only clonal cytogenetic findings are listed. Clonal aberrations were found in 15 specimens. Tetraploidy as single aberration was detected in 5 samples. Loss of the Y chromosome was seen in 8 samples, in 4 thereof together with tetraploidy.

One tumor (P1/30) contained a clone with 46,XY,−16,+der(16)[1;(16;q12;q23)], resulting in partial trisomy of 1q and partial monosomy of 16q (22). Additionally, the aberrant cells partly lost the Y chromosome. Tumor P2/5 showed a pericentromeric inversion of chromosome 9 in all the cells investigated, which is supposed to be a constitutional without pathogenetic relevance. Eight samples showed only normal male karyotypes, 46,XY.

<table>
<thead>
<tr>
<th>Biopsy</th>
<th>Age (yr)</th>
<th>Staging</th>
<th>Grading</th>
<th>Clonal karyotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/0</td>
<td>74</td>
<td>pTPN2</td>
<td>III</td>
<td>45,X−Y[=1][2]</td>
</tr>
<tr>
<td>1/2</td>
<td>69</td>
<td>pTP2, pPN2</td>
<td>II</td>
<td>45,X−Y[47]</td>
</tr>
<tr>
<td>2/5</td>
<td>57</td>
<td>pTP2, pPN2</td>
<td>II</td>
<td>46,XY,[inv(9p13q13)[10]]92,XXYY;</td>
</tr>
<tr>
<td>1/7</td>
<td>71</td>
<td>pTP2, pPN2</td>
<td>II</td>
<td>45,X−Y[91/46,XY[4]/90.XX−Y−Y[5]]</td>
</tr>
<tr>
<td>1/1</td>
<td>55</td>
<td>pTP2, pPN2</td>
<td>II</td>
<td>46,XY[32]/92,XXYY[16]</td>
</tr>
<tr>
<td>3/10</td>
<td>55</td>
<td>pTP2, pPN2</td>
<td>II</td>
<td>46,XY[32]/92,XXYY[23]</td>
</tr>
<tr>
<td>1/22</td>
<td>67</td>
<td>pTP2, pPN2</td>
<td>II</td>
<td>46,XY[32]/92,XXYY[5]</td>
</tr>
<tr>
<td>1/26</td>
<td>69</td>
<td>pTP2, pPN2</td>
<td>II</td>
<td>46,XY[32]/92,XXYY[6]</td>
</tr>
<tr>
<td>1/28</td>
<td>69</td>
<td>pTP2, pPN2</td>
<td>II</td>
<td>46,XY[32]/92,XXYY[12]</td>
</tr>
<tr>
<td>1/30</td>
<td>67</td>
<td>pTP2, pPN2</td>
<td>II</td>
<td>46,XY[32]/92,XXYY[12]</td>
</tr>
</tbody>
</table>

Table 1: Clonal cytogenetic and histopathological data of 24 biopsies from prostate carcinomas

Table 2: Clonal cytogenetic data of biopsies (taken from carcinomatous prostates) lacking carcinoma cells in histological control sections

<table>
<thead>
<tr>
<th>Biopsy</th>
<th>Age (yr)</th>
<th>Histomorphology</th>
<th>Clonal karyotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/6</td>
<td>58</td>
<td>Normal prostatic tissue</td>
<td>46,XY[8]</td>
</tr>
<tr>
<td>1/21</td>
<td>55</td>
<td>Normal prostatic tissue</td>
<td>46,XY[22]/92,XXYY[4]</td>
</tr>
<tr>
<td>2/26</td>
<td>69</td>
<td>Normal prostatic tissue</td>
<td>46,XY[6]/92,XXYY[4]</td>
</tr>
<tr>
<td>1/37</td>
<td>61</td>
<td>Normal prostatic tissue</td>
<td>46,XY[17]</td>
</tr>
<tr>
<td>1/23</td>
<td>65</td>
<td>Normal prostatic tissue</td>
<td>45,XY−Y[4]/46,XY[35]</td>
</tr>
<tr>
<td>1/24</td>
<td>66</td>
<td>Normal prostatic tissue</td>
<td>46,XY[15]</td>
</tr>
<tr>
<td>1/39</td>
<td>67</td>
<td>BNN</td>
<td>46,XY[19]</td>
</tr>
<tr>
<td>1/29</td>
<td>67</td>
<td>BNN</td>
<td>46,XY[31]/92,XXYY[5]</td>
</tr>
<tr>
<td>1/25</td>
<td>63</td>
<td>PIN</td>
<td>46,XY[14]</td>
</tr>
<tr>
<td>1/50</td>
<td>69</td>
<td>PIN</td>
<td>46,XY[34]/47,XY:+mar[4]/</td>
</tr>
<tr>
<td>1/22</td>
<td>67</td>
<td>PIN</td>
<td>46,XY[34]/47,XY:+mar[4]/</td>
</tr>
<tr>
<td>1/53</td>
<td>58</td>
<td>PIN</td>
<td>46,XY[23]</td>
</tr>
</tbody>
</table>

* Numbers in brackets, number of cells observed.

Table 2 gives a compilation of cytogenetic data from the 12 biopsies lacking carcinoma cells in control sections. In the 6 samples diagnosed as normal prostatic tissue, loss of the Y chromosome was found in one, and two contained tetraploid cells, the others showing normal male karyotypes. In three cases, BNN was detected histologically; the corresponding karyotypes were normal, except tetraploidy in P2/39. P2/53 showed histo-/cytomorphological features supposed to be premalignant (PIN) (23, 24) in the majority of cells. Less than 10% of cells were evaluated to be malignant. No clonal cytogenetic abnormality was detected. P2/50 and P2/22 contained areas with PIN as sole morphological feature. Both showed clonal aberrations, the first having gained a G-group-sized marker chromosome (Fig. 1) and the second having lost the Y chromosome.

Some of the analyzed samples revealed nonclonal aberrations, often in a very high percentage of the totally analyzed cells (Table 3). Nonclonal loss of chromosomes was the most common event. In addition, nonclonal structural abnormalities and gain of chromosomes were detected. Marked chromosome loss (lack of 1–3 chromosomes in more than 10% and at least 3 of analyzed metaphases) was found in 10 of 27 biopsies containing malignant and/or premalignant tissue. In the 14 samples with nonmalignant histology, marked chromosome loss was found in only one. Losses involved all chromosomes, except chromosomes 3 and X. Whether the chromosome losses follow a random pattern or not cannot be answered on a statistical basis because of the limited number of samples analyzed.

Examination of the metastatic iliacal lymph node LN 1/33 revealed no clonal chromosome abnormality. However, nonclonal abnormalities including marked chromosome loss and structural aberrations were detected in 51.8% (28/54) of observed cells; furthermore, one cell contained double minute chromosomes. Structural aberrations were del(2)(q27) and del(7)(p11.11). Another iliacal lymph node, LN 2/22, where metastatic changes were not detectable histomorphologically, contained cells showing 46,X−Y,+3/47,XY,+3 as clonal abnormality.

As a control we cytogenetically analyzed 5 samples of prostatic tissue derived from two males without prostatic diseases. No aberration, excepting two tetraploid clones, was seen.

DNA Flow Cytometric Studies. Twenty-three specimens were examined. In 3 cases, histological control revealed normal prostatic tissue, and no clones with aberrant ploidy were detected. One of three biopsies representing BNN areas contained a hypodiploid clone (DI, 0.91), and the other two showed normal diploid DNA content as did two biopsies with premalignant histology (PIN).

Flow cytometric measurements performed on cancerous tissue samples detected aneuploid clones in 10 cases (67%), hypodiploid ones more frequent (41%) than hyperdiploid ones (20%). Hypodiploid DNA indices ranged from 0.75 to 0.92 and hyperdiploid indices from 1.17 to 1.20. In 5 samples, aneuploidy and diploidy were detected in...
mosaicism. From 5 carcinomas, 2 biopsies per tumor were analyzed; in 3 cases the 2 biopsies showed different clonal composition.

Table 4 gives a compilation of 11 biopsies being characterized histologically, cytogenetically, and by flow cytometry. In 3 specimens clonal aberrations were detectable cytogenetically; flow cytometry yielded aberrant clonal patterns in 5 other biopsies. The presence of tetraploid cells characterized cytogenetically could not be confirmed by flow cytometry.

**FISH Studies on Paraffin-embedded Histological Sections.** As loss of the Y chromosome was the most common abnormality seen in our cytogenetic studies, FISH was used to clear up the question of its presence. In 3 carcinomas, flow cytometric measurement revealed hyperdiploidy (DI, 1.17), giving evidence that the near 3:1 ratio reflects trisomy 1 as part of the aberrant chromosomal composition. In culture, this tumor showed an atypical growth pattern with very low mitotic activity. Cytogenetic preparation yielded no informative metaphases.

The average quotient of tumor biopsy P2/30 was 2.476 (SD, 0.115). Cytogenetics detected cells with loss of Y as well as hypotetraploid ones. It cannot be decided if the abnormal quotient reflects the presence of some of these hypotetraploid cells. Flow cytometric measurements were not performed due to the little biopsy amount. Biopsy P1/30 from the same tumor showed an average quotient of 1.917 (SD, 0.254).

**DISCUSSION**

For precise assignment of genotypic changes to histomorphological features, histological characterization of control sections was performed on each biopsy which was included in our study. This was done additionally to routine histopathological diagnostics. Despite the macroscopic characterization as "intratumoral tissue," histological control sections revealed in a third of the investigated biopsies (12 of 36) no cancer. Samples from prostates with T3 carcinomas may only contain normal prostatic epithelium or benign nodular hyperplasia. This reflects one major problem in the investigation of prostatic carcinoma samples, i.e., the discrepancy between the macroscopic aspect and histology. The unexpectedly high percentage of nonmalignant histological controls in our study is supposed to be relevant for the understanding of the 78% cytogenetically normal "carcinomas" without any clonal aberration described in the literature (see references in the "Introduction"). In addition to general tumor grade and stage, focal histological control is necessary for appropriate interpretation of cytogenetic findings. Without those focal controls, we would have interpreted the cytogenetics of PIN, BHN, and normal prostatic tissue biopsies as to be correlated with the general tumor grades of II or III.

![Fig. 1. GTG-banded karyotype of a cell from biopsy 2/50 showing 47,XY,+mar. Histomorphological evaluation of 2/50 revealed prostatic intraepithelial neoplasia (PIN).](image)
Nevertheless, it should be noted that absolute congruency of histology and cytogenetics cannot be reached. In some cases the tissue brought into culture may contain the very border area of the carcinoma with tumor-free control sections.

DNA Flow Cytometry and Cytogenetics. In the last decade, much interest has been spent on DNA flow cytometric investigation of prostate cancer (26, 27). Most tumors with stage comparable to our material (pT2 N0 M0/pT3 N0 M0) were measured with tetraploidy in approximately 20 to 50% and nontetraploid aneuploidy in 4 to 10% (28, 29). Some authors favor a model of tumor progression expressed by a sequence of diploid-tetraploid-aneuploid DNA content, probably occurring simultaneously with histological dedifferentiation (30-32).

Our flow cytometric measurements detected aneuploidy in 67% of the histologically confirmed cancerous biopsies. Hypodiploid clones were more frequent than hyperdiploid ones. In the series of biopsies investigated by means of cytogenetics as well as flow cytometry, aneuploid clones were measured in six cases showing no clonal aberration cytogenetically. Interestingly, the cytogenetically aberrant clones detected in our study were all near diploid. Thus, we hypothesize that cell culture selects against highly aneuploid prostatic carcinoma cells. In a most recent publication, similar results were obtained showing a higher aneuploidy rate detected with FISH than with metaphase cytogenetics (33). In the future, DNA flow cytometry in addition to cytogenetics may help to compare and to improve cell culture techniques.

Comparing our results with the DNA flow cytometric literature, we found a higher number of aneuploid clones and no significant tetraploid ones. This may reflect differences in the methodologic approach. In contrast to the most previous studies, we used fresh biopsies instead of formalin-fixed, paraffin-embedded tissue. This enabled us to overcome some well-known difficulties of DNA histogram interpretation resulting from the irreversible cross-linking of formalin to nuclear DNA causing limited accessibility of fluorescent dyes and from methodic problems concerning isolation of nuclei from paraffin-embedded tissue (34, 35). Furthermore, most flow cytometric protocols described in the literature, focusing on the detection of tetraploidy and hyperdiploid aneuploidy, are hardly suitable for the detection of hypodiploid aneuploidy (27, 28). A more detailed discussion of our method, emphasizing methodical aspects, is published elsewhere.3

Cytogenetic Findings. Loss of the Y chromosome was found to be a frequent event occurring in 8 of 24 histologically confirmed cancerous biopsies (33%). In contrast, −Y was detected in only 1 of 9 biopsies with normal epithelium or BNH in histological controls and never in the five control samples. It, of course, cannot be ruled out whether −Y in the one biopsy histologically containing normal epithelium (P2/38) reflects the existence of a small number of carcinoma cells in the tissue brought into culture which did not appear in control sections. −Y is the most common of aberrations reported in prostate cancer. It was detected in 17 of the 35 cases with clonal chromosome changes (7, 10-13). While the number of investigated prostatic tissues (carcinoma versus noncarcinoma) is still small, our data suggest nevertheless that −Y in prostatic tissue is cancer related. This would be in accordance with an in situ hybridization study revealing −Y only in a carcinoma biopsy and not in normal prostatic tissue (36). However, our FISH study on tissue sections indicates that −Y cells may be overrepresented in culture. In our study, regional loss of the Y chromosome, as detected most recently to occur in urothelial carcinoma by using the same FISH method (37), could not be shown.

The implications of −Y on tumor biology remain unclear due to cytogenetic reports on loss of Y in normal brain (38), kidney (39, 40), and bone marrow of healthy elderly men (6). Based on the concept of clonal evolution, loss of Y appears to change additional preexisting aberrations in tumor P1/30 and the metastatic iliac lymph node LN2/22. Both showed aberrant clones (P1/30 with a der(16) marker chromosome and LN2/22 with trisomy 3) partly with and partly without −Y. We hypothesize that −Y is a secondary event in cancerogenesis of the prostate. Whether there are prognostic implications of −Y as reported for bladder carcinomas (41) cannot be answered to date.

Table 4 Comparison of cytogenetic and flow cytometric data

<table>
<thead>
<tr>
<th>Biopsy</th>
<th>Histology</th>
<th>Clonal cytogenetic aberrations</th>
<th>Nonclonal chromosome losses (%)</th>
<th>Aberrant clones detected by FCM*</th>
<th>Aberrant DNA indices</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/44</td>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/39</td>
<td>BNH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/39</td>
<td>BNH</td>
<td>92,XXYY</td>
<td>4.1</td>
<td>10.7</td>
<td></td>
</tr>
<tr>
<td>2/55</td>
<td>BNH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/53</td>
<td>PIN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/40</td>
<td>PCA</td>
<td>45,X,−Y</td>
<td>15.8</td>
<td>0.92</td>
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</tr>
<tr>
<td>1/41</td>
<td>PCA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/41</td>
<td>PCA</td>
<td></td>
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<td>1/47</td>
<td>PCA</td>
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<tr>
<td>1/51</td>
<td>PCA</td>
<td></td>
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<tr>
<td>2/51</td>
<td>PCA</td>
<td>92,XXYY</td>
<td>15.8</td>
<td>0.91</td>
<td></td>
</tr>
</tbody>
</table>

* FCM, DNA flow cytometry; PCA, prostatic carcinoma.

Fig. 2. Top, DAPI-stained nuclei in a paraffin section of a prostatic adenocarcinoma (biopsy 2/7). Bottom, the same section hybridized with chromosome-specific probes in a two-color technique (red signals: rhodamine detection for Y chromosome; green signals: fluorescence isothiocyanate detection for chromosome 1). Note the circumscribed absence of Y signals in nuclei of the glandular tumor lesion indicating microfocal loss of Y (center of the photograph).

3 K. Romanakis et al., manuscript in preparation.
Nonclonal losses were reported at first by Brothman et al. (11). In a series of 30 prostatic carcinomas analyzed, 11 showed marked chromosome loss according to the above-mentioned definition. Further evidence for the significance of nonclonal chromosome losses was given by a series of 62 cytogenetically analyzed prostatic carcinomas (13). In 16%, nonclonal numerical abnormalities in more than 10% of the metaphases were detected with loss of chromosomes occurring more often than gain.

We found marked chromosome loss in 10 of 27 histologically confirmed cancerous biopsies. To exclude preparation artefacts, attention was paid to count only metaphases without signs of preparation-induced loss of chromosomes, most of them being surrounded by intact cell membranes. The high correlation to malignancy suggests most likely that they represent a biological feature of malignant cells. Nonclonal losses are not supposed to be directly correlated to the hypodiploid clones seen in flow cytometry but may probably reflect genetic instability of tumor cells which gives rise to the development of hypodiploid clones. Cells with loss of Y perhaps can be regarded as a subgroup with a proliferation advantage in the culture system. In most cases, no cells with chromosome gains were detected, suggesting that the mechanism of chromosome loss is not mitotic nondisjunction. If anaphase lagging or other mechanisms are involved remains to be answered. However, with respect to the high frequency of latent carcinoma the question is reasonable if unspecific mechanisms, e.g., genetic instability, are involved in carcinogenesis of the prostate.

Tetraploidy was seen in carcinoma as well as in BNH, normal prostatic tissue derived from cancerous prostate and normal prostatic control samples. Statistically significant tetraploid clones were never detected by DNA flow cytometry. Hence, cytogenetic tetraploidy seems to be a nonspecific event in prostatic tissue. Nevertheless, it is possible that cytogenetics is able to detect relevant tetraploid clones occurring in small mosaicism and therefore not distinguishable by means of statistical flow cytometry and that those clones could be of importance in tumorigenesis.

**Focal Intratumoral Heterogeneity.** Intratumoral heterogeneity was revealed by all applied methods. When two cancer (or PIN) samples per tumor were analyzed, cytogenetics discovered discrepant clones in three of five cases; flow cytometric measurements showed heterogeneous clonal pattern in three of five cases too; FISH on tissue sections detected different signal quotients in two of three cases and heterogeneity concerning microfocal loss of the Y chromosome in a further one. Thus, our findings are strongly indicative for intratumoral genetic heterogeneity in prostate cancer.

There are two main hypotheses to explain this heterogeneity. (a) It could be a sign for polyclonality of prostate cancer, corresponding to the multicentricity of early carcinoma as described by Byar and Mostofi (42). (b) It could be regarded as the heterogeneity of different tumor sublines derived from a common precursor cell. In any case, our data give evidence that a single cytogenetically or flow cytometrically analyzed tumor biopsy is hardly representative for a given carcinoma.

The claim for prognostic relevance of single biopsy investigations (for example, DNA flow cytometry on fine needle biopsies) is therefore highly questionable (28).

**BNH and Premalignant Tissue.** The three investigated BNH biopsies were cytogenetically normal. One of them showed a hypodiploid clone in flow cytometry. Flow cytometric analysis of cancer-free BNH prostates will clear up the question if this finding can be regarded as related to BNH or a tumor offshoot. The first report on cytogenetics on BNH cultures was published by Brothman (10). The clonal karyotypes of the two BNH were 46,XY and 45,X,Y.

The three PIN samples showed all cytogenetic features found to be correlated with malignancy; P2/22 contained a cell clone with -Y, P2/50 showed a marker chromosome, and P1/53 showed nonclonal losses in 41.6% of the investigated metaphases. Thus, these changes seem to be early events in the development of prostate cancer.

In summary, the closely combined application of cytogenetics, FISH, and DNA flow cytometry appears as a powerful tool for the examination of prostate cancer. It allowed us to clear up reasons for the rareness of reported cytogenetically aberrant prostatic adenocarcinomas. This rareness may at least to some part be due to sampling problems, clonal selection mechanisms in the cell culture system, and intratumoral focal heterogeneity. We showed that comprehensive investigation of more than a single biopsy sample from a given tumor is warranted to elucidate genetic changes in prostatic cancer.

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**REFERENCES**


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