Aneusomies of Chromosomes 8 and Y Detected by Fluorescence in Situ Hybridization Are Prognostic Markers for Pathological Stage C (pT3N0M0) Prostate Carcinoma


ABSTRACT

In an attempt to identify new prognostic markers, we performed fluorescence in situ hybridization (FISH) ploidy analysis of tumor tissue from patients with a targeted stage and histological grade of prostate carcinoma. We identified all 227 patients from the Mayo Clinic radical prostatectomy data base who had a high histological grade pathological stage C (pT3N0M0) tumor removed between 1966 and 1987. After histological review of the paraffin-embedded specimen blocks, 181 cases were suitable for FISH analysis using chromosome enumeration probes for chromosomes 7, 8, 10, 12, X, and Y. FISH detected 80 (44%) diploid, 22 (12%) tetraploid, and 79 (44%) aneuploid tumors. The common aneusomies were of chromosomes 7 and 8, which were present in 51 (28%) and 46 (25%) tumors, respectively. Aneusomies of chromosomes 10, 12, X, and Y were observed in 11 (6%), 15 (8%), 12 (7%), and 16 (9%) tumors, respectively.

FISH aneuploid tumors showed a trend of more frequent systemic prostate cancer progression than nonaneuploid tumors (P = 0.006). For individual chromosome anomalies, gains of chromosome 8, aneusomy of chromosome 8, and aneusomy of chromosome Y correlated highly with systemic cancer progression (P = 0.006, 0.013, and 0.021, respectively). Gains of chromosome Y and aneusomy of chromosome Y were associated with an increased prostate cancer death rate (P < 0.001 for both). Multivariate analysis showed that gains of chromosome 8 and aneusomy of chromosome Y were significant independent "predictors" of systemic cancer progression (P = 0.008) and cancer death (P < 0.001), respectively. These results demonstrate that aneuploidy and specific aneusomies detected by FISH are potential markers for a poor prognosis in histological high-grade pathological stage C (pT3N0M0) prostate carcinoma.

INTRODUCTION

Prostate adenocarcinoma has extensive variability in clinical behavior. Accurate prediction of individual tumor progression probability and patient survival is a major goal of current prostate cancer research. Parameters such as clinical and pathological stage, histological grade, and pretreatment serum PSA are conventionally used to help predict the prognosis for individual patients with clinically localized prostate carcinoma (1). Newer factors, such as DNA content ploidy analysis using FCM or static image analysis, can help refine prognostic risks (2). However, it is recognized that FCM or static image ploidy analysis cannot detect small changes in DNA content or chromosome number.

Interphase cytogenetic analysis FISH, using chromosome enumeration probes, is useful for detecting numerical chromosome alterations in a variety of solid tumors (3, 4). Previous studies from this laboratory have demonstrated that FISH analysis is more sensitive than FCM for detecting aneuploidy of radical prostatectomy specimens, and that aneusomies of chromosomes 7 and 8 are associated with higher histological grade and advanced pathological stage (5–7). These results encouraged us to evaluate the hypothesis that nonrandom numerical chromosome alterations detected by FISH are useful markers for tumor progression and patient prognosis. As a preliminary trial, we performed a case-control FISH study using two groups of 25 patients with clinically localized prostate carcinoma who were matched for clinicopathological features but who showed distinctively different prognoses (8). This study demonstrated that overall aneuploidy and aneusomy of chromosome 7 were significantly associated with relatively rapid prostate cancer death.

We now have performed an inclusive retrospective FISH tumor analysis of patients with a single defined pathological stage and histological grade of prostate adenocarcinoma. We targeted histological high-grade pathological stage C (pT3N0M0) prostate cancers treated by radical prostatectomy. Clinical tumor progression rates and cause-specific survival of the patients with this grade and stage of prostate carcinoma are difficult to predict. In an attempt to identify useful new prognostic markers for these cancers, FISH analysis was carried out using chromosome enumeration probes for six different chromosomes.

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3 The abbreviations used are: PSA, prostate-specific antigen; FCM, flow cytometry; FISH, fluorescence in situ hybridization.
MATERIALS AND METHODS

Study Design. Of 1786 patients in the Mayo Clinic radical prostatectomy data base whose tumors were surgically removed between 1966 and 1987, 637 (36%) were found to have pathological stage C (pT3N0M0) prostate cancer. All patients had pelvic lymphadenectomies concurrently and did not have metastatic deposits in the pelvic lymph nodes. Pathological stage C (pT3) disease denotes prostate cancers that have perforated the prostatic capsule and involve periprostatic tissues, seminal vesicles, membranous urethra, and bladder neck (1). Among this large group, 227 patients who had histological high-grade tumors and adequate clinical follow-up were selected as our study group. Tumor grade was determined by the Mayo nuclear morphological grading system, and tumors of grade 3 or 4 were considered “high-grade tumors” for this study (9).

The clinicopathological data available for these patients included patient age, tumor volume, seminal vesicle involvement (if present), preoperative serum PSA level (which became available in 1987), flow cytometric DNA ploidy, and adjuvant therapy (if applied). Tumor volume was calculated as described previously (10), and serum PSA level was measured with a monoclonal solid-phase two-site immunoradiometric assay (Hybritech, Inc., San Diego, CA). FCN analysis on formalin-fixed paraffin-embedded prostatectomy specimens was performed with the same method as previously described (5). Systemic prostate cancer progression and prostate cancer death were used as clinical end points. Systemic progression was defined as clinical evidence of distant metastatic disease and ascertained by positive findings on bone scan or other radiological imaging tests.

The patient list was randomized, and the following tumor specimen FISH analyses were performed without knowledge of the clinicopathological findings and survival data of the patients.

Tissue Preparations. For each case, a surgical pathologist previously had identified a single prostate specimen block which contained the highest histological grade prostate cancer for FCM ploidy analysis. These paraffin-embedded tumor blocks were sectioned and histologically reviewed, and only those specimens with ≥30% cancer cells were used in this investigation. After review, 181 cases were suitable for FISH analysis. Three adjacent 50-μm tumor tissue sections were used for FISH analyses. Tissue deparaffinization for FISH on isolated nuclei was carried out using the previously described technique (8). In brief, sections were washed with 2 ml Histo-Clear (National Diagnostics, Atlanta, GA) three times. The tissue was then dehydrated in 100% ethanol and rinsed in water. Tissues were digested in pepsin solution for 2.5 h at 37°C. Following hybridization, the slides were washed in 50% formamide/2× SSC, 2× SSC, and 2× SSC/NP40. Nuclei were counterstained with 1 μg/ml 4',6-diamidino-2-phenylindole dihydrochloride.

The FISH signals were counted with a Zeiss Axioplan microscope equipped with single-pass filters (150191 and 150291; Vysis). The hybridization signals for each probe in 300 interphase nuclei were counted. To minimize sampling error, two individuals counted signals in 150 nuclei on each half of the hybridized area. All morphologically intact nuclei (apparently truncated or overlapping nuclei were excluded) within that area were evaluated. When an obvious discrepancy was observed between these independent counts by two individuals, a third individual enumerated signals on the same slide without information of the previous results, and the two most concordant results of the three were used for analysis. The total percentage of nuclei containing one, two, three, four, five, six, seven, eight, and more than eight signals was determined for each chromosome.

Criteria for FISH Ploidy and Aneusomy. To define ploidy and aneusomy objectively, previously defined criteria were used (6–8). In brief, based upon the mean ± 3 SDs of centromere copy numbers in benign prostate hyperplasia samples and an inspection of prior prostate carcinoma FISH results, the following conservative cutoff values were established.

1. A tumor was classified as tetraploid if the autosomal average of the percentage of nuclei with four signals was ≥6%.
2. An abnormal monosomy (or nullisomy for sex chromosomes) required ≥12% of the nuclei to contain one (or zero) FISH signal.
3. An abnormal autosomal trisomy was required to fulfill two criteria: (a) ≥7% nuclei contain three FISH signals and (b) the ratio of three signal nuclei:four signal nuclei be ≥2:3. The latter criterion is necessary because in tetraploid tumors the three-signal population may be elevated as a result of inefficient hybridization.
4. Sex chromosome gain or tetraploidy required ≥12% nuclei with two FISH signals.
5. To distinguish sex chromosomal gain from tetraploidy, the former required that the ratio of the percentage of nuclei with two sex chromosome signals to the average autosomal four signal percentage be ≥2:1
6. Abnormal hypertetrasomy required that the sum of the percentage of nuclei with ≥5 signals for any autosome or ≥3 signals for any sex chromosome be ≥6%.
7. Abnormal monosomy/nullisomy, trisomy, or hypertetrasomy defined a tumor as aneuploid. Otherwise, the tumor was classified as apparently diploid or apparently pure tetraploid based on the overall average autosomal tetrasomy.
Table 1 shows typical examples of a FISH diploid, tetraploid, and two aneuploid tumors. The FISH diploid tumor (case 1) had an average autosomal tetrasomic population of 2% and, based on the criteria listed above, contained no apparent chromosome centromere anomaly. The FISH tetraploid tumor (case 9) had an average autosomal tetrasomy of 24.7% and, except for the increased tetrasomy for each autosome and increased disomy for each sex chromosome, contained no apparent chromosome centromere anomaly. The first FISH aneuploid tumor (case 40) had an average autosomal tetrasomy of 2.5% and, based on the criteria listed above, was observed to have abnormal centromere signal distributions for chromosomes 7 and 8 (aneusomies, +7 and −8). The second FISH aneuploid tumor (case 208) had an average autosomal tetrasomy of 8.2%, which met the tetraploid criteria, but also was observed to have abnormal centromere signal distributions for chromosomes 8, X, and Y (aneusomies, +8, +X, and +Y).

Statistical Analysis. After evaluating all FISH signal count results and classifying FISH anomalies according to the criteria described above, their association with clinicopathological and prognostic parameters of the patients studied were analyzed statistically. Among FISH anomalies, we independently tested overall FISH aneuploidy and aneusomies (pooling gains and losses) of chromosomes 7 and 8 and gains of chromosomes 7, 8, 10, 12, X, and Y against clinical end points. Gains of chromosomes 7, 8, 10, 12, X, and Y were also examined. Chromosomal losses were not analyzed separately because of the small numbers of cases with this type of numeric anomaly.

Comparison of patient groups with respect to baseline data was done using the χ2 or rank sum test. Unless otherwise noted, all tests were two-sided with α level of 0.05. Survival curves from date of prostatectomy to the end points of cause-specific death and systemic cancer progression were estimated using the Kaplan-Meier method. Comparison of survival curves was done using the log rank test. Multivariate analyses for systemic cancer progression and prostate cancer death were performed using the Cox proportional hazards model. Candidate predictors were age, seminal vesicle involvement, estimated tumor bulk, adjuvant therapy, FCM DNA ploidy, FISH ploidy (aneuploid versus nonaneuploid), aneusomies of chromosomes 7, 8, 10, 12, X, and Y, and gains of chromosomes 7, 8, 10, 12, X, and Y. Stepwise backwards variable selection was used with α level of 0.01.

Preliminary evaluation of FISH ploidy with three groups found similar outcomes for diploid and tetraploid cases with respect to systemic cancer progression and cause-specific death. In view of this, the FISH ploidy was analyzed as aneuploid versus nonaneuploid. A Bonferroni-type correction of the P values was done to adjust for this post hoc pooling of the data. The univariate P values from the log rank test for FISH ploidy were multiplied times 2, the number of ways we might have considered grouping the data (i.e., diploid versus nondiploid or aneuploid versus nonaneuploid).

RESULTS

Ploidy and Numeric Chromosome Alterations Detected by FISH. Table 2 summarizes the distribution of FISH results in the 181 tumor specimens studied. According to the criteria described above, FISH detected 80 (44%) diploid, 22 (12%) tetraploid, and 79 (44%) aneuploid tumors.
Table 2 Distribution of FISH results in 181 patients with histological high-grade pathological stage C (pT3N0M0) prostate carcinoma.

<table>
<thead>
<tr>
<th>FISH findings</th>
<th>No. of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ploidy</td>
<td></td>
</tr>
<tr>
<td>Diploid</td>
<td>80 (44.2)</td>
</tr>
<tr>
<td>Tetraploid</td>
<td>22 (12.2)</td>
</tr>
<tr>
<td>Aneuploid</td>
<td>79 (43.6)</td>
</tr>
<tr>
<td>Aneusomy of chromosome</td>
<td>Gain</td>
</tr>
<tr>
<td>7</td>
<td>50 1</td>
</tr>
<tr>
<td>8</td>
<td>39 7</td>
</tr>
<tr>
<td>10</td>
<td>9 2</td>
</tr>
<tr>
<td>12</td>
<td>15 0</td>
</tr>
<tr>
<td>X</td>
<td>12 0</td>
</tr>
<tr>
<td>Y</td>
<td>12 4</td>
</tr>
<tr>
<td>Presence of hypertetrasomy</td>
<td></td>
</tr>
<tr>
<td>No. of aneusomic chromosomes</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>102 (56.4)</td>
</tr>
<tr>
<td>1</td>
<td>40 (22.1)</td>
</tr>
<tr>
<td>2</td>
<td>23 (12.7)</td>
</tr>
<tr>
<td>3</td>
<td>9 (5.0)</td>
</tr>
<tr>
<td>4-6</td>
<td>7 (3.9)</td>
</tr>
</tbody>
</table>

Aneusomies were of chromosome 7 and 8, which were observed in 51 (28%) and 46 (25%) tumors, respectively. Aneusomies of chromosomes 10, 12, X, and Y were found in 11 (6%), 15 (8%), 12 (7%), and 16 (9%) tumors, respectively. Gains of chromosome relative to the inferred stemline ploidy level were more common than losses (Table 2). Thirteen (7%) aneuploid tumors showed hypertetrasomic (≥5 signals) anomalies. Forty (22%) aneuploid tumors had one, 23 (13%) had two, and 16 (9%) had ≥3 aneusomic chromosomes.

**Correlation with Clinicopathological Characteristics.**

The overall mean age at surgery was 65 years. Of the 181 studied patients, seminal vesicles were invaded in 110 (61%) patients. Eighteen (60%) of 30 evaluable patients showed >10 ng/ml preoperative serum PSA level. Standard FCM DNA ploidy analysis of the formalin-fixed paraffin-embedded radical prostatectomy specimens was possible for 167 patients and tumors had one, 23 (13%) had two, and 16 (9%) had ≥3 aneusomic chromosomes.

**Correlation with Systemic Cancer Progression and Patient Survival.**

Table 4 summarizes relationships between FISH results and patient prognosis. The median clinical follow-up of the 181 patients was 6.8 (range, 0.4–21) years. During the period of follow-up, 57 (31%) patients had systemic prostate cancer progression. The percentage of patients without systemic cancer progression was 77%, 62%, and 35% at 5, 10, and 15 years, respectively (Fig. 1A). Aneuploid cases showed a trend of more frequent systemic cancer progression, with a systemic progression-free probability at 10 years of 53% compared to 68% for nonaneuploid cases (adjusted P = 0.060; Fig. 1B). Individual aneusomy of chromosome 8 correlated with more frequent systemic cancer progression (P = 0.013, Table 4). Moreover, cases with gain of chromosome 8 showed a stronger association with systemic progression (P = 0.006, Table 4), with a systemic progression-free rate of 44% at 10 years compared to 68% for cases without gain of chromosome 8 (Fig. 1C). Aneusomy of chromosome Y correlated with systemic progression (P = 0.021, Table 4; Fig. 1D), but when cases with and without chromosome Y gain were compared, this correlation no longer remained statistically significant (P = 0.15, Table 4).

Thirty-five (19%) patients died from prostate cancer during the period of follow-up. Overall prostate cancer-specific survival was 91%, 79%, and 49% at 5, 10, and 15 years, respectively (Fig. 2A). Prostate cancer-specific survival of patients with FISH aneuploid tumors was 77% at 10 years compared to 80% of patients with FISH nonaneuploid tumors (P = 0.38, Table 4; Fig. 2B). Gains of chromosome 8 did not show significant association with prostate cancer death (P = 0.24, Table 4; Fig. 2C). Both aneusomy and gains of chromosome Y strongly correlated with prostate cancer death (P < 0.001 for both; Table 4). Ten-year cancer-specific survival of patients with aneusomy was 52%; in contrast, that of patients with the normal chromosome Y number was 81% (Fig. 2D).

No other chromosomal aneusomies or chromosomal gains correlated with systemic cancer progression or prostate cancer death. In addition, neither the presence of hypertetrasomy nor the number of aneusomic chromosomes correlated with these clinical endpoints.

To examine the strength of the association of FISH anomalies with systemic cancer progression and prostate cancer death after adjustment for the clinicopathological variables, multivariate analysis was performed using the Cox model described in “Materials and Methods.” A gain of chromosome 8 was the only significant independent predictor of systemic cancer progression among all of the examined variables (P = 0.008). Aneusomy of chromosome Y was the only significant independent predictor of prostatic cancer death (P < 0.001).

**DISCUSSION**

At this institution, about one-third (36%) of the patients undergoing radical prostatectomy for clinically localized pros-
tate cancer from 1966 to 1987 were found to have pathological progression rates and survival for the patients with pathological tumors (1). A previous study of pathological stage C patients histological grade tumors, but not for patients with high-grade because in the previous reports cases with a tumor of higher stage C (pT3NoMo) tumors (1). It is difficult to predict clinical to that found in our previous FISH analyses for clinically tumors (12). Mosome enumeration probes was routinely applicable to archi- value of numeric chromosome alterations detected by FISH in prostate carcinoma. The overall distribution of nuclear ploidy and aneusomies found in this investigation was essentially iden-

To the best of our knowledge, this is the first comprehen-
sive retrospective survival analysis to evaluate the prognostic value of numeric chromosome alterations detected by FISH in prostate cancer or any solid tumor. FISH analysis using chromosome enumeration probes was routinely applicable to archival paraffin-embedded specimens of pathological stage C (pT3) prostate carcinoma. The overall distribution of nuclear ploidy and aneuploidy found in this investigation was essentially identical to that found in our previous FISH analyses for clinically localized prostate cancers (most of them are of pathological stage B or C; Refs. 5–7), except for a slightly higher frequency of aneuploidy and aneuploidy. This finding may be reasonable because in the previous reports cases with a tumor of higher grade or advanced pathological stage showed more frequent aneuploidy (6, 7), and this study targeted only high histological grade pathological stage C disease. Our previous studies also demonstrated that ploidy detected by FISH is consistent with that detected by FCM and a degree of numerical chromosome alterations corresponds well to FCM DNA index values (5–7). However, these studies (5–7) also demonstrated that FISH is more sensitive and accurate than FCM for the detection of aneuploidy. These observations were confirmed by this investigation.

FISH aneuploidy tumors showed a trend of more frequent systemic cancer progression and higher preoperative PSA level than nonaneuploid tumors. Serum PSA level has been reported to help predict clinical outcome (13). Unfortunately, PSA levels were not routinely determined prior to 1987, making more detailed analysis of potential associations impossible.

To date, the prognostic relevance of genetic alterations on chromosome Y has not been studied in prostate cancer. In this study, both gains and aneuploidy of chromosome Y were associated with an increased prostate cancer death rate. Moreover, aneuploidy Y was a significant independent predictor of cancer death. In previous standard cytogenetic studies, loss of chromosome Y was reported as one of the most common clonal aberrations in prostate cancer (14, 15) and in a variety of other neoplasms including leukemia, renal cell carcinoma, and brain tumors (15–18). Loss of chromosome Y was also detected in non-neoplastic brain and kidney tissue and in bone marrow cells of healthy elderly men. Therefore, its significance as a cancer-related alteration has been controversial (15, 19). However, recent interphase FISH studies, including ones from our institution, have demonstrated more frequent gains of chromosome Y in prostate cancer (6, 8, 20, 21). This alteration is apparently confined to cancerous prostatic epithelial cells (20, 21). On the basis of these literature and our new findings, we believe that gains as well as general aneuploidy of chromosome Y is a relatively common genetic alteration of prostate adenocarcinoma and may be a useful new marker for poor prognosis in pathological stage C prostate cancer. The role of chromosome Y aneuploidy itself in cancer progression is not clear from the experiments we performed. Although the presence of aneuploidy Y correlated with progression, the presence of chromosome Y gain did not. This observation suggests that chromosome Y loss may be associated with progression. Indeed, three of the eight progression events observed for the 16 patients with aneusomy Y occurred in the 4 patients with loss of the Y. However, this correlation of loss of the Y with progression did not reach

**Table 3** Clinicopathological characteristics and FISH results of 181 patients with histological high-grade pathological stage C (pT3NoMo) prostate carcinoma

<table>
<thead>
<tr>
<th>Clinicopathological characteristics</th>
<th>Nonaneuploida</th>
<th>Aneuploid</th>
<th>P</th>
<th>Normal chromosome 8</th>
<th>Aneusomy 8</th>
<th>P</th>
<th>Normal chromosome y</th>
<th>Aneusomy y</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>65</td>
<td>64</td>
<td>0.26</td>
<td>65</td>
<td>64</td>
<td>0.28</td>
<td>65</td>
<td>64</td>
<td>0.28</td>
</tr>
<tr>
<td>Range (yr)</td>
<td>50–78</td>
<td>52–75</td>
<td></td>
<td>50–78</td>
<td>52–75</td>
<td></td>
<td>50–78</td>
<td>54–69</td>
<td></td>
</tr>
<tr>
<td>Tumor bulk (%)</td>
<td>48</td>
<td>63</td>
<td>0.066</td>
<td>53</td>
<td>56</td>
<td>0.77</td>
<td>54</td>
<td>56</td>
<td>0.92</td>
</tr>
<tr>
<td>Seminal vesicle involvement (%)</td>
<td>59</td>
<td>65</td>
<td>0.43</td>
<td>59</td>
<td>70</td>
<td>0.18</td>
<td>61</td>
<td>69</td>
<td>0.52</td>
</tr>
<tr>
<td>Preoperative serum PSA level (%)</td>
<td>47</td>
<td>82</td>
<td>0.063</td>
<td>58</td>
<td>67</td>
<td>0.71</td>
<td>59</td>
<td>100</td>
<td>0.41</td>
</tr>
<tr>
<td>FCM ploidy (%)</td>
<td>9</td>
<td>23</td>
<td>0.010</td>
<td>11</td>
<td>26</td>
<td>0.018</td>
<td>14</td>
<td>29</td>
<td>0.14</td>
</tr>
<tr>
<td>Adjuvant therapy (%)</td>
<td>46</td>
<td>49</td>
<td>0.66</td>
<td>50</td>
<td>41</td>
<td>0.33</td>
<td>47</td>
<td>50</td>
<td>0.83</td>
</tr>
</tbody>
</table>

*Only aneusomies that have significant correlation with clinical endpoints (see Table 4) are listed.
*Nonaneuploid group consists of diploid (n = 80) and tetraploid cases (n = 22).
*Comparing the distribution of each parameter among FISH results using the χ² or rank sum (for age) test.
*One hundred fifty cases had tumor volume available.
*Thirty cases treated during 1987 were available. Only one case with aneusomy Y was available.
*One hundred sixty-seven cases had FCM ploidy available.
*Hormonal and/or radiotherapy within 90 days after prostatectomy.
Table 4  FISH results and clinical end points for 181 patients with histological high-grade pathological stage C (pT3N0M0) prostate carcinoma

| FISH result                                      | Systemic cancer progression-free | Prostate cancer-specific survival | p
|-------------------------------------------------|----------------------------------|----------------------------------|---
| Overall Ploidy                                   |                                  |                                  |   
| Nonaneuploid*                                   | 68 (8)                           | 80 (60)                          |   
| Aneuploid                                       | 53 (9)                           | 77 (5)                           | 0.38*

| Individual chromosome alterations                |                                  |                                  |   
| 7: Normal                                       | 59 (8)                           | 77 (5)                           |   
| Aneusomic                                       | 67 (6)                           | 79 (5)                           | 0.060*
| No gain                                         | 59 (8)                           | 77 (5)                           |   
| Gain                                            | 67 (8)                           | 85 (6)                           | 0.53*
| 8: Normal                                       | 67 (6)                           | 76 (7)                           |   
| Aneusomic                                       | 47 (11)                          | 79 (5)                           | 0.013
| No gain                                         | 68 (6)                           | 80 (5)                           |   
| Gain                                            | 44 (11)                          | 73 (8)                           | 0.24
| 10: Normal                                      | 65 (5)                           | 80 (4)                           |   
| Aneusomic                                       | 33 (25)                          | 66 (16)                          | 0.46
| No gain                                         | 62 (6)                           | 80 (4)                           |   
| Gain                                            | 58 (19)                          | 61 (18)                          | 0.20
| 12: Normal                                      | 62 (6)                           | 79 (5)                           |   
| Aneusomic (gain)*                               | 53 (14)                          | 79 (11)                          | 0.28
| No gain                                         | 62 (6)                           | 80 (4)                           |   
| Gain                                            | 51 (13)                          | 63 (15)                          | 0.75
| X: Normal                                       | 61 (6)                           | 80 (4)                           |   
| Aneusomic (gain)*                               | 71 (14)                          | 63 (15)                          | 0.82
| Y: Normal                                       | 62 (6)                           | 81 (4)                           |   
| Aneusomic                                       | 51 (13)                          | 52 (13)                          | <0.001
| No gain                                         | 62 (6)                           | 81 (4)                           |   
| Gain                                            | 62 (15)                          | 54 (16)                          | <0.001

* Kaplan-Meier progression-free rate or survival estimate (SE) at 10 years. There were 16 patients at risk for systemic progression and 23 at risk for prostate cancer-specific death at 10 years.

* *P values from log rank test comparing overall progression-free rate or survival curves.

* Nonaneuploid group consists of diploid (n = 80) and tetraploid tumors (n = 22).

* Adjusted P value equals to two times nominal P value.

* Losses were not observed for chromosomes 12 and X.

statistical significance. It may be that aneusomy of chromosome Y is simply a marker of poorer prognosis tumors and is not pathogenetically related to their worse clinical behavior.

Gains and aneusomy of chromosome 8 correlated with systemic cancer progression, and, moreover, gain of chromosome 8 was a significant independent predictor for systemic progression. Previous FISH studies from our research group have shown that gains of chromosome 8 are common in clinically localized prostate cancers and are associated with higher tumor grades and advanced pathological stages (6, 7). Molecular genetic alterations in chromosome 8 have been implicated in the carcinogenesis of multiple tumors (22, 23). Homozygous deletion and frequent allelic loss of 8p22 loci in prostate cancers have been reported, suggesting the presence of a putative tumor suppressor gene in this region (24). Additionally, multiplication of 8q has been reported to often accompany the allelic loss in 8p in prostate cancer (24) and hepatocellular cancer (25). Recent FISH studies of prostate cancers have confirmed that a loss of signals at 8p22 often correlate with a gain of centromere 8 signals (26–28). A likely genetic mechanism underlying both the FISH and molecular genetic observations is the acquisition of multiple isochromosomes 8q in tumor cells. Interestingly, amplification of the myc oncogene, which is located on 8q24, is reported to be correlated with a shorter disease-free interval and overall survival for the patients with breast carcinoma (29). Furthermore, amplification of DNA sequences from 8q24 was recently observed in prostate cancer patients with lymph node metastasis or recurrent disease using FISH with a region-specific probe (30). In this study, gain of chromosome 8 correlated with systemic cancer progression but not increased cancer death. The reason for this finding is unclear. It is possible that the gain of chromosome 8 or 8q has importance for metastatic potential, but that a subpopulation of tumor cells with this change requires additional genetic alterations to become more aggressive (e.g., become androgen independent).

Gain of chromosome 7 was the most common numeric chromosome alteration found in this study, but showed no apparent individual diagnostic relevance. In previous FISH studies from this and other institutions, aneusomy of chromosome 7 correlated with a less favorable phenotype (high tumor grade and advanced pathological stage; Refs. 7, 31, and 32). Also, gain of chromosome 7 (trisomy 7) has been reported as a consistent anomaly in other solid tumors such as urinary bladder, brain, colon, and kidney (4, 33), suggesting a common locus for carcinogenesis. We performed a preliminary case-control FISH study with pathological stage B and C patients who died from metastatic prostate cancer within 3 years after radical prostatectomy. We found that aneusomy of chromosome 7 is a
Fig. 1  Systemic cancer progression-free rate after radical prostatectomy for the 181 patients with histological high-grade pathological stage C (pT3N0M0) prostate cancer. A, overall; B, according to FISH aneuploidy (log rank test; P = 0.060); C, according to gains of chromosome 8 (P = 0.006); and D, according to aneusomy of chromosome Y (P = 0.021).

Fig. 2  Cancer-specific survival after radical prostatectomy for the 181 patients with histological high-grade pathological stage C (pT3N0M0) prostate cancer. A, overall; B, according to FISH aneuploidy (log rank test; P = 0.38); C, according to gains of chromosome 8 (P = 0.24); and D, according to aneusomy of chromosome Y (P < 0.001).
nonrandom alteration that correlates with clinically aggressive prostate cancer (8). For this current investigation, we used the same FISH methodology and criteria for FISH aneusomy as in the preliminary case-control study. The reason for the discordant results regarding aneusomy of chromosome 7 is unclear. One possibility could be the difference of the patient groups studied. The previous case-control study included pTaN0M0 tumors and used patients with a “very poor prognosis” (e.g., patient death within 3 years after surgery; Ref. 8). In addition, the definition of gain and aneusomy used in our studies could be fulfilled by a small subpopulation of tumor cells. Thus, it is possible that a biological character of the residual majority of tumor cells influences the patient prognosis. The criteria for gain and aneu-

somy used were established based on the consistent results of our previous studies (6–8). We believe that these criteria are reasonable for this study and that changing these criteria to better stratify patients according to clinical outcome is statistically inappropriate (34). Because of the consistent incidence of the aneusomy of chromosome 7 in prostate cancer (6–8, 32, 33), additional studies to evaluate the prognostic relevance of this common numeric alteration in prostate carcinoma will be important.

In this retrospective analysis, FISH signals were enumerated at least by two independent individuals, and all investiga-
tions were carried out without knowledge of the clinicopathological characteristics and survival data of the patients studied. We believe that this study was performed with acceptable minimum bias, and that aneuploidy and gains and aneusomies of chromosomes 8 and Y detected by FISH are new potential markers for a poor prognosis in pathological stage C prostate carcinoma. These findings may be of important practical use, since we have reported a methodology to perform rapid FISH chromosome analysis on pretreatment prostate needle biopsy core specimens that can subsequently be used for routine histopathological examination (7). The pretreatment detection of cytogenetic markers associated with systemic cancer progression and cancer death described above may prove useful for selecting patients who would benefit from early aggressive systemic adjuvant treatment, while minimizing the therapeutic burden to those who do not require it.

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Aneusomies of chromosomes 8 and Y detected by fluorescence in situ hybridization are prognostic markers for pathological stage C (pt3N0M0) prostate carcinoma.


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