Clinical Significance of Alterations of Chromosome 8 in High-Grade, Advanced, Nonmetastatic Prostate Carcinoma

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Background: Chromosome 8 alterations, including loss of 8p21–22 and gain of 8q24, are commonly observed in prostate carcinoma. We examined whether these alterations are associated with poor prognosis in prostate cancer.

Methods: We used dual-probe fluorescence in situ hybridization and DNA probes for 8p22 (lipoprotein lipase gene), centromere 8 (8cen), and 8q24 (c-myc gene) to determine the corresponding copy numbers in tumor samples from 144 patients with high-grade, advanced (stage III) prostate carcinoma. Cox models were used for multivariate analysis of systemic progression or patient death from prostate cancer. All statistical tests are two-sided.

Results: We classified the 8p22, 8cen, and c-myc copy number as normal, loss, and gain. An additional increase (AI) category of c-myc relative to the centromere copy number (i.e., overrepresentation and amplification of c-myc) was also used. Alterations of 8p22 were not statistically significantly associated with either systemic progression or patient death. Alterations of c-myc were associated with both systemic progression (P = .024) and patient death (P = .039); AI of c-myc showed the poorest outcome. We also evaluated the prognostic relevance of the combined 8p22–8cen–c-myc loci anomaly pattern for the following six patterns: normal–normal–normal, loss–any 8cen–normal, loss–gain–gain, gain–gain–gain, non-loss–any 8cen–AI, and loss–any 8cen–AI, where any 8cen is normal, loss, or gain of the chromosome 8 centromere. Patients with the loss–any 8cen–AI pattern had earlier systemic progression (P = .009) and earlier cause-specific death (P = .013) than did patients with other patterns.

Conclusions: Genetic alterations of chromosome 8 appear to accumulate in parallel with the progression of prostate carcinomas. AI of the c-myc gene, especially with loss of 8p22, appears to be associated with poor patient prognosis. [J Natl Cancer Inst 1999;91:1574–80]

Several chromosomal and genetic aberrations have been found in prostate carcinoma. Loss of heterozygosity of markers mapped to chromosome region 8p21–22 is common in human prostate cancer (1–4). Loss of 8p is suggested to be an early event in tumorigenesis because prostate intraepithelial neoplasia has also been shown to have frequent loss of heterozygosity of 8p (5). These results suggest that a gene or genes mapped to 8p are involved in the early stage of prostate cancer development. Studies (6–9) have also shown band 8q24 to be commonly gained in prostate carcinoma, often accompanied by 8p loss. The c-myc gene, a well-known regulator of cell proliferation and programmed cell death, maps to this region (10). We have previously demonstrated (11) that the c-myc gene is overrepresented in prostate carcinoma with lymph node metastases and that the frequency of overrepresentation increases from prostate intraepithelial neoplasia to primary carcinoma and metastases. Thus, unlike loss of 8p, overrepresentation of c-myc appears to be associated with prostate cancer progression.

The development of a fluorescence in situ hybridization (FISH) technique has allowed the investigation of numerical chromosomal anomalies and genetic alterations on a cell-by-cell basis within a region of interest (12,13). For the detection of numerical chromosome alterations in solid tumors, FISH analysis of interphase cells has been proven to be a more...
sensitive and reliable method than Southern blot hybridization (14) and polymerase chain reaction (15–17).

Clinically aggressive behavior is associated with an accumulation of genetic aberrations in solid tumors, such as colon cancer (18) and urinary bladder cancer (19). Similar multiple genetic changes may occur in prostate carcinoma. Prostate cancer is a leading cause of death of men in the United States, so the identification of patients whose tumor is destined to progress rapidly is a major goal of current research. Unfortunately, within a cohort of men with a single grade and stage of prostate cancer, there are few markers of clinical aggressiveness. High-stage prostate carcinoma often has multiple genetic abnormalities, often involving 8p and 8q. To determine whether 8p loss and/or 8q gain predict a poor prognosis in prostate cancer, we used dual-probe FISH to investigate the copy number changes of cancer, we used dual-probe FISH to investigate the copy number changes of cancer, we used dual-probe FISH to investigate the copy number changes of cancer, we used dual-probe FISH to investigate the copy number changes of cancer.

MATERIALS AND METHODS

Patients

A comprehensive analysis of allelic imbalance of chromosome arms 7q, 8p, 16q, and 18q has been previously performed on a large cohort of high-grade stage III prostate carcinomas (20). All 227 patients underwent radical prostatectomy and pelvic lymphadenectomy during the period from 1966 through 1987, and metastases were not identified. Thus, all patients had pathologic stage T3N0M0 (tumor–node–metastasis) cancer (21). One hundred fifty-seven specimens from this cohort contained an adequate number of tumor cells in the paraffin blocks for FISH analysis. The overall mean age of these patients at surgery was 66 years (range, 53–79 years). The order of patients in the list was randomized, and FISH analyses were performed on these 157 tumor specimens by individuals who did not have knowledge of the clinicopathologic findings and survival data of the patients.

As described below, FISH was successfully performed on 144 (91.7%) specimens. The FISH data on these patients were compared with the corresponding Gleason scores. We divided the tumors into three Gleason score groups of 4–6, 7, and 8–10, as reported previously (10). Because we selected predominantly high-grade tumors, only 16 (11.1%) of the 144 prostate carcinomas had a Gleason score of 4–6. Of the remaining 128 tumors, 64 (44.4% of 144 patients) had a Gleason score of 7 and 64 (44.4% of 144 patients) had a Gleason score of 8–10. Seminal vesicle involvement was observed in 90 (62.5%) patients. Surgical margins were positive for carcinoma in 58 (40.3%) patients. Thirty-one (21.5%) and 24 (16.7%) patients postoperatively received adjuvant hormonal therapy and radiotherapy, respectively, whereas two patients (1.4%) received both therapies. Standard flow cytometry DNA ploidy analysis was possible for 133 samples; 45 (33.8%) were diploid, 70 (52.6%) were tetraploid, and 18 (13.5%) were aneuploid. The preoperative serum concentration of prostate-specific antigen (the assay for which became available in 1987) was not included.

Follow-up data were obtained by a nurse who contacted the patients annually by telephone or in writing as a part of the formal, ongoing Mayo Clinic Radical Prostatectomy Tumor Registry (22). Briefly, systemic prostate carcinoma progression and prostate carcinoma-specific death were used as clinical endpoints. Systemic progression was defined as biochemical evidence of systemic cancer and was ascertained by positive findings on bone scan or other radiologic imaging tests. Whether the patient’s death was caused by prostate cancer was ascertained at the time of the patient’s death by a combination of death certificate review, contact with the primary physician, and discussion with the patient’s family, if necessary. The mean follow-up of these patients was 7.7 years (median = 7.5 years).

Of the 144 patients on whose samples FISH was successful, 14 received hormonal therapy before prostatectomy. These patients were excluded from the prognostic studies. Among the remaining 130 patients, 35 (26.2%) had systemic disease progression and 28 (21.5%) died of prostate cancer.

We compared the distribution of clinical variables between the patients whose samples provided FISH results and the patients whose paraffin blocks were not available or whose samples did not provide FISH results. There was no statistically significant difference in DNA ploidy status, pathologic stages, postsurgical adjuvant treatment, 10-year progression-free survival, or 10-year overall survival between these two groups of patients (data not shown).

Informed consent was obtained from all of the patients for the use of their follow-up information. This study was approved by the Mayo Clinic Institutional Review Board.

Tissue Preparation

For each patient, a surgical pathologist previously had identified a single prostate specimen block that contained the highest (worst) histologic grade of prostate carcinoma. Fifteen tissue sections (5 μm thick) were sliced from each paraffin-embedded tumor block and mounted on glass slides. The first tissue section was stained with hematoxylin–eosin to ascertain the region of interest.

Dual-Probe FISH With Centromere 8 and Locus-Specific Probes

FISH is described elsewhere (11). Briefly, tissue sections were deparaffinized, dehydrated, treated with microwave radiation in 10 mM citric acid (pH 6.0) for 10 minutes, digested with pepsin (4 mg/mL in 0.9% NaCl [pH 1.5]) for 12 minutes at 37 °C, rinsed in 2× standard saline citrate (SSC) (pH 7.2) at room temperature, and air-dried. Dual-probe hybridization then was performed with a centromere 8 probe (chromosome enumeration probe 8 [CEP8]; Vyysis, Inc., Downers Grove, IL) and with a locus-specific probe, either an 8p22 probe (LPL gene; Vyysis, Inc.) or an 8q24.1 probe (c-myc; Vyysis, Inc.). Probes and target DNA were codenatured at 80 °C for 2 minutes, annealed at 50 °C for 30 minutes, and then incubated at 37 °C overnight. After hybridization, samples were washed in a solution of 1.5 M urea and 0.1× SSC (pH 7.2) at 45 °C for 30 minutes. Tissue sections were equilibrated in 2× SSC for 5 minutes at room temperature. Nuclei were then counterstained with 4,6-diamidino-2-phenylindole and antifade compound p-phenylenediamine.

Three hundred nonoverlapping interphase nuclei from a focus of benign epithelium and adenocarcinoma were counted for each probe with a Diaplan microscope (Leitz, Wetzlar, Germany) equipped with a triple-pass filter. By use of the hematoxylin–eosin-stained slide of the adjacent section as a reference, the same dominant tumor focus was evaluated for each probe. In some cases, there were variations in FISH findings within one tumor focus. In these samples, the cancer focus with the primary Gleason pattern was evaluated. Nuclei from stromal elements were not enumerated. Locus-specific probe (8p22 or c-myc) and CEP8 signals were enumerated for each nucleus.

Criteria for FISH Anomalies

A normal value study was performed by enumerating 8p22, c-myc, and CEP8 signals in histologically normal prostatic epithelial nuclei of 10 patients, as described previously (11) (data not shown). With the use of results from the normal value study and an inspection of the distribution of each FISH signal among the carcinoma foci, we categorized the 8p22, c-myc, and CEP8 copy number status of a tumor focus as normal, gain, and loss. In addition, the category of additional increase (AI) of c-myc copy number relative to the centromere copy number was also used. This category contains overrepresentation (e.g., duplication, triplication, etc.) and amplification of c-myc. The threshold values for these categories were chosen to minimize the detection of false-positive changes. The normal category required less than 10% of epithelial nuclei with three or more signals and less than 55% of epithelial nuclei, with zero or one signal for an applied probe. The gain category required 10% or more of epithelial nuclei with three or more signals for an applied probe. The category of loss of CEP8 required 55% or more of epithelial nuclei with zero or one signal for CEP8. The category of loss of 8p22 required the overall mean 8p22/CEP8 ratio of less than 0.85. The category of loss of c-myc required the overall mean c-myc/CEP8 ratio of less than 0.90. The AI category was applied only to c-myc and required the overall mean c-myc/CEP8 ratio of more than 1.3 and 10% or more of epithelial nuclei with three or more signals for c-myc.

Statistical Analysis

The frequency and distribution of FISH anomalies in prostate carcinoma were compared by use of the Pearson χ² test and Student’s t test. The relationships of FISH anomalies with Gleason scores were evaluated with the Pearson χ² test. Kaplan–Meier curves and the logrank test were used to estimate systemic progression-free survival or cause-specific death. Univariate comparisons of survival curves were done with the logrank test. All risk ratios for disease progression and survival were estimated by use of the multivariate Cox proportional hazards model. Variables in the model included findings, Gleason score, seminal vesicle involvement, surgi-
al margin status, use of postoperative adjuvant therapy, and aneuploid flow cytometry ploidy pattern. All statistical tests were two-sided with an \( \alpha \) level of 0.05. A \( P \) value of less than 0.05 is considered statistically significant in all tests.

**RESULTS**

**Summary of FISH Anomalies in 144 Stage III Prostate Cancer Specimens**

Of 157 tumors subjected to FISH analysis, FISH was performed successfully on 144 (91.7%) for c-myc/CEP8 and 143 (91.1%) for 8p22/CEP8.

By applying the cutoff values described in the “Materials and Methods” section, we defined 78 (54.2%) of 144 prostate carcinomas as having a gain of c-myc gene copy number. Of these 78 tumors, 50 (34.7% of 144) had a gain of 8cen and an equivalent gain of c-myc and 28 (19.4% of 144) had an AI of c-myc alone. Among the 28 tumors with an AI of c-myc alone, 16 (11.1% of 144) had a gain of 8cen and an AI of c-myc and 12 (8.3% of 144) had an AI of c-myc alone. The group with an AI of c-myc alone contained two tumors that had loss of 8cen and 10 tumors that had no apparent anomaly of 8cen. No loss of c-myc was found in this study. Two carcinomas (1.4%) designated as having loss of 8cen had a high c-myc/CEP8 ratio due to loss of 8cen, without an actual increase (three or more) of c-myc signals per nucleus.

Tumors with loss of 8p22 were clearly separated from those with no loss of 8p22 by the 8p22/CEP8 ratio cutoff of 0.85. One hundred nine (76.2%) of 143 prostate carcinomas were defined as having 8p22 abnormalities. Among these tumors, 89 (62.2% of 143) had a loss of 8p22 and 20 (14.0% of 143) had a gain of 8p22. We observed no apparent homozygous deletion of 8p22 in this study.

A similar CEP8 copy number status for c-myc and 8p22, defined in the dual-probe hybridization experiments, was observed, and the CEP8 FISH classifications were concordant between the two experiments. Sixty-six (45.8%) of the 144 patients had tumors with a gain of 8cen, and four (2.8%) had tumors with a loss of 8cen.

Table 1, A, summarizes 8p22, 8cen, and c-myc FISH results for all 144 patients. We classified FISH anomalies as one of 11 patterns, which represent all of the genetic alterations of chromosome 8 that occurred in this cohort of patients. The first pattern normal–normal–normal (i.e., normal FISH findings for 8p22–8cen–c-myc) was observed in 31 (21.5%) tumors. Results from 113 (78.5%) tumors with abnormal FISH findings were distributed among the other 10 patterns.

**Association With Gleason Score**

Table 1, B, shows that there was no statistically significant association between Gleason score and loss of 8p22 (\( P = .11 \)). However, the Gleason score was statistically significantly associated with a gain of 8p22, a gain of 8cen, a gain of c-myc, and an AI of c-myc (\( P = .01 \), \( P < .01 \), \( P = .03 \), and \( P = .02 \), respectively). When we considered the combined FISH results for 8p22, 8cen, and c-myc, the percentage of tumors with a Gleason score of 8–10 increased from 29.0% to 36.4% to 50.0% to 63.6% for the dominant FISH anomaly patterns normal–normal–normal, loss–normal–normal, loss–gain–gain, and loss–gain–AI, respectively.

**Association With Systemic Cancer Progression and Patient Survival**

Alterations in 8p22 were not statistically significantly associated with systemic cancer progression (\( P = .63 \)) (Fig. 1, A) or patient death (\( P = .14 \)) (Fig. 1, B). Ten-year progression-free survival

### Table 1. Pertinent chromosome 8 fluorescence in situ hybridization (FISH) data for 144 patients with stage III prostate carcinoma*

**A) Classification of FISH findings based on 8p22, 8cen, and c-myc status**

<table>
<thead>
<tr>
<th>Group</th>
<th>8p22</th>
<th>8cen</th>
<th>c-myc</th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>31</td>
<td>21.5</td>
</tr>
<tr>
<td>2</td>
<td>Loss</td>
<td>Normal</td>
<td>Normal</td>
<td>33</td>
<td>22.9</td>
</tr>
<tr>
<td>3</td>
<td>Loss</td>
<td>Loss</td>
<td>Normal</td>
<td>2</td>
<td>1.4</td>
</tr>
<tr>
<td>4</td>
<td>Loss</td>
<td>Gain</td>
<td>Gain</td>
<td>34</td>
<td>23.6</td>
</tr>
<tr>
<td>5</td>
<td>Gain</td>
<td>Gain</td>
<td>Gain</td>
<td>15</td>
<td>10.4</td>
</tr>
<tr>
<td>6</td>
<td>Normal</td>
<td>Normal</td>
<td>AI</td>
<td>3</td>
<td>2.1</td>
</tr>
<tr>
<td>7</td>
<td>Loss</td>
<td>Normal</td>
<td>AI</td>
<td>7</td>
<td>4.9</td>
</tr>
<tr>
<td>8</td>
<td>Loss</td>
<td>Loss</td>
<td>AI</td>
<td>2</td>
<td>1.4</td>
</tr>
<tr>
<td>9</td>
<td>Loss</td>
<td>Gain</td>
<td>AI</td>
<td>11</td>
<td>7.6</td>
</tr>
<tr>
<td>10</td>
<td>Gain</td>
<td>Gain</td>
<td>AI</td>
<td>5</td>
<td>3.5</td>
</tr>
<tr>
<td>11</td>
<td>—†</td>
<td>Gain</td>
<td>Gain</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>144</td>
<td>100</td>
</tr>
</tbody>
</table>

**B) Correlation of FISH abnormalities with Gleason score**

<table>
<thead>
<tr>
<th>Gleason score</th>
<th>No. of patients</th>
<th>8p22†</th>
<th>8cen§</th>
<th>c-myc</th>
<th>No. (%) of patients with indicated FISH anomaly</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Loss</td>
<td>Gain</td>
<td>Normal</td>
<td>Gain</td>
</tr>
<tr>
<td>4–6</td>
<td>16</td>
<td>7 (21)</td>
<td>8 (9)</td>
<td>0 (0)</td>
<td>13 (18)</td>
</tr>
<tr>
<td>7</td>
<td>64</td>
<td>17 (50)</td>
<td>40 (45)</td>
<td>7 (35)</td>
<td>36 (49)</td>
</tr>
<tr>
<td>8–10</td>
<td>64</td>
<td>10 (29)</td>
<td>41 (46)</td>
<td>13 (65)</td>
<td>25 (34)</td>
</tr>
<tr>
<td>Two-sided P‡</td>
<td>.11</td>
<td>.01</td>
<td>&lt;.01</td>
<td>.03</td>
<td>.02</td>
</tr>
</tbody>
</table>

*AI = additional increase; 8cen = centromere 8.
†Not determined.
‡One specimen without 8p22 data was excluded.
§Four specimens with loss of 8cen were excluded.
¶All comparisons are with the normal FISH group.
Fig. 1. Kaplan–Meier survival curves for 130 patients with stage III prostate carcinoma. The number of patients at risk at 0, 5, and 10 years is also shown. A) Progression-free survival by 8p22 status. Ten-year progression-free survival rates for groups with the pattern a normal 8p22, a loss of 8p22, and a gain of 8p22 were 77.1% (95% confidence interval [CI] = 63.6%–93.6%), 72.9% (95% CI = 63.3%–83.8%), and 69.7% (95% CI = 48.0%–100.0%), respectively. B) Cause-specific survival by 8p22 status. Ten-year cause-specific survival rates for groups with the pattern a normal 8p22, a loss of 8p22, and a gain of 8p22 were 90.3% (95% CI = 80.5%–100.0%), 76.4% (95% CI = 67.1%–86.9%), and 94.1% (95% CI = 83.6%–100.0%), respectively. C) Progression-free survival by c-myc status. Ten-year progression-free survival rates for groups with the pattern a normal c-myc, a gain of c-myc, and an additional increase (AI) of c-myc were 80.6% (95% CI = 70.9%–91.6%), 71.3% (95% CI = 58.0%–87.8%), and 57.5% (95% CI = 40.5%–81.6%), respectively. D) Cause-specific survival by c-myc status. Ten-year cause-specific survival rates for groups with the pattern a normal c-myc, a gain of c-myc, and an AI of c-myc were 87.6% (95% CI = 79.4%–96.7%), 83.3% (95% CI = 72.7%–95.5%), and 63.7% (95% CI = 46.4%–87.6%), respectively. E) Progression-free survival by the 8p22–8cen (i.e., centromere 8)–c-myc loci pattern. Ten-year progression-free survival rates were 78.2% (95% CI = 64.2%–93.3%) for normal–normal–normal (Table 1, A; group 1), 82.9% (95% CI = 70.3%–97.8%) for loss–any 8cen–normal (groups 2 and 3), 78.4% (95% CI = 64.4%–95.4%) for loss–gain–gain (group 4), 60.8% (95% CI = 36.2%–100.0%) for gain–gain–gain (group 5), 85.7% (95% CI = 63.3%–100.0%) for non-loss–any 8cen–AI (groups 6 and 10), and 45.5% (95% CI = 26.6%–77.7%) for loss–any 8cen–AI (groups 7, 8, and 9), respectively. Any 8cen is for normal, loss, or gain of chromosome 8 centromere. F) Cause-specific survival by 8p22–8cen–c-myc loci pattern. Ten-year cause-specific survival rates were 89.3% (95% CI = 78.5%–100.0%) for normal–normal–normal, 86.2% (95% CI = 74.5%–97.8%) for loss–any 8cen–normal, 82.0% (95% CI = 68.8%–97.8%) for loss–gain–gain, 92.3% (95% CI = 78.9%–100.0%) for gain–gain–gain, 100.0% (95% CI = 100.0%–100.0%) for non-loss–any 8cen–AI, and 47.1% (95% CI = 27.3%–81.3%) for loss–any 8cen–AI.
rates for the groups with a normal 8p22, a loss of 8p22, and a gain of 8p22 were 77.1%, 72.9%, and 69.7%, respectively. Similarly, 10-year cause-specific survival rates for the groups with a normal 8p22, a loss of 8p22, and a gain of 8p22 were 90.3%, 76.4%, and 94.1%, respectively.

An AI of c-myc was statistically significantly associated with an increased probability of systemic progression ($P = .024$) (Fig. 1, C) and cause-specific death ($P = .039$) (Fig. 1, D). Ten-year progression-free survival rates for the groups with a normal c-myc, a gain of c-myc, and an AI of c-myc were 80.6%, 71.3%, and 57.5%, respectively. Similarly, 10-year cause-specific survival rates for the groups with a normal c-myc, a gain of c-myc, and an AI of c-myc were 87.6%, 83.3%, and 63.7%, respectively.

Multivariate analysis indicated that an AI of c-myc was a statistically significant independent predictor of systemic progression but was of only borderline statistical significance for cause-specific death. The risk ratio for an AI of c-myc was 2.4 (95% confidence interval $[CI] = 1.1–5.1$; $P = .021$) for systemic progression and was 1.8 (95% CI = 0.8–4.2; $P = .14$) for cause-specific death when adjusted for Gleason grade, seminal vesicle involvement, margin positivity, and adjuvant therapy (data not shown). Further adjustment for DNA content was done by use of DNA probes for 8p22 (LPL gene), 8cen, and gain 8q.

When the aneuploid flow cytometry ploidy pattern was included in the multivariate analyses, the pattern loss–any 8cen–AI had risk ratios of 2.7 (95% CI = 1.0–7.5; $P = .048$) and 3.4 (95% CI = 1.2–9.9; $P = .024$) for systemic progression and cause-specific survival, respectively (Table 2, model 2).

**Discussion**

By dual-probe FISH with the use of DNA probes for 8p22 (LPL gene), 8cen, and c-myc gene on 144 prostate carcinomas at stage III, we found the following: 1) Prostate carcinomas have frequent genetic abnormalities of chromosome 8 (78.5%), especially a loss of 8p22; 2) a gain of chromosome 8 and an AI of c-myc are statistically significantly associated with Gleason score; and 3) an AI of c-myc was associated with systemic progression and patient survival, but a loss of 8p22 was not associated with systemic progression and patient survival.

On the basis of the frequency of FISH anomaly patterns observed in this study, we hypothesize that the accumulation of gene aberrations in prostate carcinoma occurs primarily in three steps (Fig. 2, thick arrows). In the first step, 8p22 is deleted. Mutation or a small deletion of a gene or genes on 8p that is not detectable by the 8p22 FISH probe also may take place. Previous studies of 8p loss in prostate intraepithelial neoplasia and in prostate carcinoma (2,20,23,24) support this idea. Second, a whole chromosome 8 is gained (perhaps the chromosome 8 that suffered the first 8p22 loss). Third, 8q is gained [possibly one of the chromosome 8 undergoes isochromosome 8q formation, which will simultaneously delete 8p and gain 8q (6–9,11,12)]. A smaller region, including the c-myc gene, may also be overrepresented or amplified.

**Table 2. Relative risk of metastasis and cause-specific death estimated with the multivariate Cox model in 130 patients with stage III prostate carcinoma**

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Systemic cancer progression</th>
<th>Cause-specific death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model 1</td>
<td>Model 2</td>
</tr>
<tr>
<td></td>
<td>Risk ratio (95% CI)†</td>
<td>$P^*$</td>
</tr>
<tr>
<td>Loss–any 8cen–AI†</td>
<td>3.3 (1.5–7.3)</td>
<td>.003</td>
</tr>
<tr>
<td>Gleason score per unit increase</td>
<td>1.0 (0.8–1.4)</td>
<td>.95</td>
</tr>
<tr>
<td>Seminal vesicle involvement</td>
<td>2.4 (1.1–5.2)</td>
<td>.027</td>
</tr>
<tr>
<td>Positive surgical margin status</td>
<td>1.2 (0.5–2.9)</td>
<td>.68</td>
</tr>
<tr>
<td>Use of postoperative adjuvant therapy</td>
<td>0.4 (0.2–0.9)</td>
<td>.038</td>
</tr>
<tr>
<td>Aneuploid FCM ploidy pattern</td>
<td>— —</td>
<td>— —</td>
</tr>
<tr>
<td>No. of patients</td>
<td>130</td>
<td>119§</td>
</tr>
<tr>
<td>No. of events</td>
<td>35</td>
<td>30</td>
</tr>
</tbody>
</table>

*CI = confidence interval; 8cen = centromere 8; AI = additional increase; FCM = flow cytometry.
†All $P$ values are two-sided.
‡Loss–any 8cen–AI (n = 18) versus others (n = 112). Any 8cen is for normal, loss, or gain of chromosome 8 centromere.
§Of the 130 patients, standard FCM DNA ploidy analysis was possible for 119.
The genetic alterations of chromosome 8 accumulated in a fashion that paralleled the clinical progression of prostate carcinomas. For example, the Gleason score increased as the number of FISH anomalies increased. Systemic progression and cause-specific survival of patients with prostate carcinoma were also statistically significantly poorer as the tumors proceeded along the major pathway (see Figs. 1 and 2). Of particular note, the 10 patients with the pattern loss–gain–AI had a 10-year progression-free survival of only 30.0%. Multivariate analyses showed that the pattern loss–gain–AI 8cen–AI was a statistically significant independent prognostic factor with the highest risk ratios for systemic cancer progression and cause-specific patient survival. These findings support the primary genetic pathway that we propose in Fig. 2.

This study suggests that the c-myc gene is a marker for the malignant potential of a prostate carcinoma. Overexpression of the c-myc gene has been found in prostate carcinoma (25,26), and we have previously shown (11) that substantial amplification of the c-myc gene is strongly associated with immunohistochemical evidence of the c-myc protein overexpression. Overexpression of c-myc protein has been hypothesized to cause degradation of p27Kip1, leading to activation of cyclin E/cyclin-dependent kinase 2 and cell proliferation (27,28). It has recently been shown (29–32) that the level of p27Kip1 is associated with Gleason score, tumor recurrence, and patient survival with prostate carcinoma. A study with the use of in vivo transduction of prostate cancer cells with antisense c-myc (33) demonstrated that tumor growth was reduced by suppressing c-myc protein. Thus, these observations suggest that overexpression of c-myc deregulates the control of cell growth, resulting in proliferation of prostate carcinoma cells. This overexpression is most often mediated through an increased c-myc gene copy number (11).

Of 130 patients with a follow-up, 35 had systemic progression and 28 died of prostate cancer after a curative surgical operation. This result suggests that these patients already had clinically undetectable metastases before the surgery. The patients whose tumor had an AI of c-myc had rapid progression and died early of cancer, indicating that the AI of c-myc enhanced the proliferation of the metastasized tumor cells. If this is the case, it is natural to speculate that the late systemic progressions at 10–12 years, observed in the patients whose prostate carcinoma had a gain of c-myc (see Fig. 1, C and D), may result from an AI of c-myc that occurred as a new genetic event in the metastatic tumor cells. Unfortunately, it is difficult to obtain specimens from late metastatic lesions to test this hypothesis. Van Den Berg et al. (8) observed amplification of 8q DNA sequences, including 8q24, in three (75%) of four metastatic lymph node lesions, but they observed this amplification in four (9%) of 44 primary prostate carcinomas. Our previous study (11) reported more frequent amplification of the c-myc gene in metastatic foci (21%) than in primary foci (8%). Thus, c-myc gene status may predict whether a metastatic prostate cancer focus progresses or not.

In addition, some patients whose tumor had a normal or a gain of c-myc had systemic progression, suggesting that metastasis of prostate carcinoma could occur without amplification of the c-myc gene and that there may be other genes involved in triggering metastasis (34).

Management of stage III prostate carcinoma is still a major clinical challenge (35). As previous studies (36,37) indicated, our study also demonstrated the superiority of FISH to flow cytometry in identifying a subset of tumors with an aggressive nature. A FISH study with c-myc may allow early cancer detection for a patient whose prostate carcinoma, especially a possible metastatic focus, is destined to progress rapidly. Postoperative systemic adjuvant therapy may benefit the patient with prostate carcinoma positive for an AI of c-myc.

We selected the c-myc probe because c-myc is overexpressed and 8q24.1 is often amplified in prostate cancer (6,11). It is possible that another gene of importance for prostate carcinoma progression lies within 8q24. For example, prostate stem cell antigen is frequently overexpressed in high-grade and high-stage prostate cancer. The prostate stem cell antigen gene has been recently cloned and is mapped to 8q24 (38). There may be other genes in 8q24 that are critical for prostate carcinogenesis. Even so, our study strongly suggests that the pattern loss–gain 8cen–AI anomaly is a useful marker for prostate carcinoma progression. Because we had only 20 (13.9%) patients with this pattern, additional studies with larger sample sizes are necessary to confirm our results.

In conclusion, our FISH results suggest that genetic alterations of chromosome 8 are statistically significantly
associated with clinicopathologic characteristics of stage III prostate carcinoma. Substantial amplification of the c-myc gene, especially with loss of 8p22, appears to predict systemic progression and poor patient prognosis and may justify an early adjunctive treatment for these patients.

REFERENCES


