Loss of p53 and c-myc Overrepresentation in Stage $T_{2-3}N_{1-3}M_0$ Prostate Cancer are Potential Markers for Cancer Progression

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To determine whether genetic changes are markers of cancer progression and patient survival in Stage $T_{2–3}N_{1–3}M_0$ prostatic carcinoma, we compared 26 patients who died of tumor relapse after prostatectomy and lymphadenectomy (case group) with 26 matched patients who were alive at the time of the matched case's death (control group). Nine unmatched cases were also included in this study. In 37 cases, paired primary tumors (119 foci) and lymph node metastases (114 foci) were available for study. Fluorescence *in situ* hybridization (FISH) with centromere-specific probes for chromosomes 7, 8, and 17 and region-specific probes for D7S486 (7q31), c-myc (8q24), LPL (8p22), and p53 (17p13) was performed on available primary carcinomas and lymph node metastases. In primary tumor foci, +7q31, −8p22, +c-myc, substantial additional increases of myc (AI-c-myc), and −p53 were observed in 65%, 74%, 43%, 29%, and 31% of foci, respectively. AI-c-myc was strongly associated with higher cancer Gleason score ($P = .003$). Heterogeneity of genetic changes was frequently observed among multiple cancer foci. Lymph node metastases of prostate cancer usually shared genetic changes with paired primary tumors. In addition, the genetic change pattern with −8p, +c-myc or AI-c-myc, +7q, and +p53 was slightly higher in lymph node metastases (22%) than in primary tumors (6%) ($P = .08$). In matched case and control patients, simultaneous gain of 7q31 (+7q31) and CEP7 (+CEP7) was identified in 59% and 68% of specimens for case and control groups, respectively ($P = .48$). Loss of 8p22 (−8p22) was identified in 77% and 69% of specimens for case and control groups, respectively ($P = 1.0$). Simultaneous gain of c-myc (+c-myc) and CEP8 (+CEP8) without overt additional increase of c-myc copy number relative to CEP8 copy number, was identified in 38% and 54% of specimens for case and control groups, respectively ($P = .27$). AI-c-myc was identified in 54% and 23% of specimens for case and control groups, respectively (odds ratio = 3.0, $P = .06$). Loss of p53 (−p53) was identified in 46% and 15% of specimens for case and control groups, respectively (odds ratio = 4.0, $P = .04$). Our results indicate that FISH anomalies are very common in both primary tumors and lymph node metastases of Stage $T_{2–3}N_{1–3}M_0$ prostate cancer; that AI-c-myc is associated with higher cancer Gleason score; that AI-c-myc and −p53 are associated with prostate cancer progression and are potential markers of survival in Stage $T_{2–3}N_{1–3}M_0$ prostate cancer; and that lymph node metastases usually have similar or additional genetic changes compared with primary tumors, and multiple lymph node metastases usually have similar genetic changes.

KEY WORDS: p53, 8p22, 7q31, c-myc, FISH, Metastasis, Prostate.

Mod Pathol 2002;15(1):35–44

Prostate cancer is the most common malignancy and the second leading cause of cancer death in men in the United States (1). Patients with pelvic lymph node metastases of prostatic carcinoma, but no evidence of systemic progression are commonly classified as having Stage $T_{2–3}N_{1–3}M_0$ prostate cancer (2). These cancers have an indeterminate natural history (3). Choosing different types of therapy (radiation, surgery, or hormonal therapy) for these

VOL. 15, NO. 1, P. 35, 2002 Printed in the U.S.A.
Date of acceptance: September 27, 2001.
This work was supported by a P20 grant from the NIH (CA 58225; to RBJ, DGB, and MML) and by a grant from Vysis (Downers Grove, IL; to RBJ).
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patients is controversial, and the clinical importance of bilateral pelvic lymphadenectomy for these patients is also debatable (3). Furthermore, genetic changes in the lymph node metastases, especially in the multiple lymph node metastases of prostate cancer, are poorly understood (4). An understanding of the genetic events that accompany the progression of prostatic adenocarcinoma and the subsequent development of metastases may be useful for prevention, early detection, and treatment (4).

Molecular genetic studies have identified multiple genetic changes in prostate cancers (5, 6). These include loss or gain of specific regions or whole chromosomes, gene amplification, and structural alterations that lead to overexpression or activation of oncogenes or inactivation of putative tumor suppressor genes (5, 6). For example, frequent allelic loss of the LPL gene (8p22) in prostate cancer suggests that a tumor suppressor gene (or genes) is located in this region (7–11). Likewise, the c-myc gene (8q24) is commonly overrepresented or amplified in advanced and recurrent prostate cancer, suggesting that amplification of this oncogene (or a neighboring gene) may be critical for the progression of this cancer (12–14). We have recently shown that concurrent overrepresentation of c-myc and loss of LPL independently predicts a poor overall survival and progression-free survival in men with stage $pT_3N_0M_0$ prostate cancer (15). Inactivation of the p53 gene has been observed in localized prostate cancer; however, the role of the p53 gene in advanced prostatic carcinoma is poorly understood (16–18). Our previous studies of primary prostatic carcinoma have shown that allelic imbalance on locus D7S486 (7q31) is strongly associated with cancer progression, lymph node metastases, and cancer-specific death (19, 20). This indicates that the region(s) at 7q31 may play an important role in the development of regional lymph node metastases and further systemic progression.

Fluorescence *in situ* hybridization (FISH) analysis of interphase nuclei using centromere-specific and region-specific probes detects numeric chromosomal anomalies and genetic alterations (21). Applying FISH to histologic sections of primary cancers and lymph node metastases allows comparison of the genetic changes from multiple foci of each (12, 22).

We performed a case-control study of genetic changes at regions of 7q31 (D7S486), 8p22 (LPL), 8q24 (c-myc), and 17p13 (p53), and numerical chromosomal anomalies in multiple foci of primary cancer and lymph node metastases. Our objectives were to determine whether any alterations can be markers of survival in Stage $T_{2–3}N_{1–3}M_0$ prostate cancer; to study intraglandular and intratumoral genetic heterogeneity; and to evaluate the relationship between multiple primary prostatic carcinoma foci and lymph node metastases.

MATERIALS AND METHODS

Patient Selection and Histopathological Evaluation

The study was a nested case-control design from a cohort of 645 patients with pathological Stage $T_{2–3}N_{1–3}M_0$ who had undergone radical retropubic prostatectomy and bilateral pelvic lymphadenectomy between 1982–1994 at the Mayo Clinic. Cases included 26 patients who died of prostate cancer within 10 years following prostatectomy. For each case, a matched control was selected from patients alive at the time of the case's death (*i.e.*, controls were selected from the risk set). Using this method, it was possible for a patient to be a control at one point and later become a case. This occurred four times, resulting in 48 unique patients for the case-control study, designed to evaluate the effect of genetic anomalies on death caused by prostate cancer. Table 1 summarizes the clinical characteristics of the 26 matched pairs in case and control groups. Matching of cases to controls was done with respect to age, pathological stage, Mayo pathological grade, and adjuvant hormonal treatment. Average follow-up time was 5.9 years for the cases and 10.7 years for the controls. Five unmatched cases and 4 unmatched controls with Stage $T_{2–3}N_{1–3}M_0$ prostate cancer were also included (in addition to the 48 patients matched above) in analysis relating the presence of genetic anomalies to tumor characteristics.

All prostate specimens were formalin fixed and paraffin embedded. For each case, representative blocks were selected for all primary cancer foci and positive lymph nodes after pathologic confirmation. In 37 cases, matched primary tumor specimens and lymph node metastases materials were available for study. In 18 cases, only the primary tumors were available, and in 2 cases, only the lymph node metastases were available. Ten serial 5-μm sections were prepared from each block, and the 1st and 10th sections were stained by routine hematoxylin and eosin and examined by two pathologists. Areas with benign epithelium, primary cancer, and metastatic cancer were outlined on the remaining eight un-

TABLE 1. Comparison of Matching Factors for 26 Pairs of Matched Cases and Controls

	Case Group (n = 26)	Control Group (n = 26)
Year of surgery, median (range)	1985 (1982–1992)	1985 (1982–1992)
Age at surgery, median (range)	62.5 (45–77)	62.5 (51–77)
Primary tumor Mayo grade (%)		
2	15	15
3	73	77
4	12	8
Adjuvant hormonal therapy (%)		
No	12	8
Yes	88	92

stained sections for FISH analysis. Gleason score was determined for each focus of cancer, and pathologic stage for each case was assigned using the TNM system (2, 23). Patients ranged in age from 56 to 70 years (mean, 63 years). Pathologic tumor stages were $T_{2-3}N_{1-3}M_0$ (TNM, 1992 revision[42]). One hundred nineteen foci of primary prostatic carcinoma were identified in 55 cases (mean, 2.2 foci per case). Multifocality (≥2 foci per case) of carcinoma was noted in 41 cases (66.7%). The Gleason scores of the 119 primary cancer foci were 4–6 (26 foci), 7 (43 foci), and 8–10 (50 foci). One hundred fourteen foci of lymph node metastases were identified in 39 cases (mean, 2.9 foci per case). Usually, the tumor cells within the same metastatic cancer focus showed similar histologic characteristics (data not shown).

FISH with Chromosome Enumeration Probes and Region-Specific Probes

Briefly, tissue sections were deparaffinized, dehydrated, treated with microwave procedure in 10 mM citric acid (pH 6.0) for 10 minutes, digested in pepsin solution (4 mg/mL in 0.9% NaCl, pH 1.5) for 10 minutes at 37° C, rinsed in 2× SSC at room temperature for 5 minutes, and air dried. Previous studies showed a high frequency of genetic changes on 7q31, 8p22, 8q24, and 17p13. Thus, for this study, we chose directly-labeled fluorescent DNA locus-specific probes (LSP) for the regions of 7q31 (D7S486), 8p22 (LPL), 8q24 (c-myc), 17p13 (p53), and for the centromere regions of chromosomes 7, 8, and 17 (CEP7, CEP8, and CEP17, respectively; Vysis, Downers Grove, IL). Dual-probe hybridization was performed on the serial 5-μm sections using a SpectrumGreen (SG)-labeled CEP8 probe, together with a SpectrumOrange (SO)-labeled 8p22 (LPL) probe, for a SG-labeled CEP8 probe with a SO-labeled 8q24 (c-myc) probe, a SG-labeled CEP7 probe with a SO-labeled 7q31 (D7S486) probe, and an SG-labeled CEP17 probe with a SO-labeled 17p13 (p53) probe. Probes and target DNA were denatured simultaneously in an 80° C oven for 5 minutes, and each slide was incubated at 37° C overnight. Posthybridization washes were performed in 1.5 M urea/0.1× SSC at 45° C for 30 minutes and in 2× SSC at room temperature for 2 minutes. Nuclei were counterstained with 4,6-diamidino-2-phenylindole and antifade compound *p*-phenylenediamine.

The number of FISH signals was counted with a Zeiss Axioplan microscope equipped with a triple-pass filter (I02–104–1010; Vysis). Two researchers (JQ, KH) independently carried out all investigations without knowledge of survival data of the patients studied. For each probe, 300 nonoverlapping interphase nuclei from foci of benign epithelium and adenocarcinoma were counted. Nuclei from stromal elements were not enumerated. The region containing the dominant Gleason pattern in each cancer focus was analyzed by FISH. An average signal ratio was calculated for each dual-probe hybridization by dividing the mean copy number of red (region-specific) with the mean copy number of green (centromeric) signals. Typical tabulated data for a representative tumor focus from case 20 for the c-myc/CEP8 probe pair is illustrated in Table 2. The rows in the table demonstrate the percentage of nuclei with different numbers of CEP8 signals. The nuclei with 0–1 and ≥3 CEP8 signals defined those cells with apparent loss (−CEP8) and gain of CEP8 (+CEP8), respectively. The columns in the table demonstrate the percentage of nuclei with different numbers of c-myc signals. The nuclei with 0–1 and ≥3 c-myc signals defined those cells with apparent loss (−c-myc) and gain of c-myc (+c-myc), respectively. Finally, a mean c-myc/CEP signal ratio was calculated for this focus. Similarly, a mean LSP/CEP ratio was calculated for each locus-specific probe for each focus. Similar variables (−CEP, +CEP, −LSP, +LSP, and the mean LSP/CEP ratio) can be used to determine whether the chromosomal centromere and/or chromosomal regions are gained or lost. Importantly, normal ranges for each of these variables can be established by evaluating apparently normal prostate epithelium (see Results).

TABLE 2. Distribution of c-myc and CEP8 Copy Numbers in a Prostate Cancer Focus from Case 20

CEP8 Copy Number	c-myc Copy Number (%)						
	0	1	2	3	4	?5	Total
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	0.0	24.7	7.3	0.3	0.0	0.0	32.3
2	0.0	7.0	58.0	0.7	0.0	0.0	65.7
3	0.0	0.0	0.4	1.3	0.0	0.0	1.7
4	0.0	0.0	0.0	0.0	0.3	0.0	0.3
?5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total %	0.0	31.7	65.7	2.3	0.3	0.0	100.0

Mean c-myc/CEP8 ratio = 1.01; average c-myc signals/nucleus = 1.71; average CEP 8 signals/nucleus = 1.70; +CEP8 = 2.0%, −CEP8 = 32.3%; +c-myc = 2.6%; −c-myc = 31.7%.

Statistical Analysis

Comparisons of FISH anomalies between the primary tumor and the metastasis and between groups of cancer Gleason score were done based on tumor foci (233 foci in 57 patients) using generalized estimating equations, which take into account the potential within-patient correlation. The probability of a specific anomaly being present was modeled as a function of type of foci (primary *versus* metastasis), or foci Gleason score. Conditional logistic regression, which takes into account the case-control matching, was used to test association of genetic anomalies or patterns with death caused by prostate cancer. These models were fit using a stratified Cox model (PHREG in SAS software). For ease of interpretation, distributions of risk factors between cases and controls were presented using unmatched tables. All tests were two-sided with significance level α set to .05.

RESULTS

Normal Value Study

To accurately ascertain the copy number alterations of the chromosomes 7, 8, and 17 centromeres and chromosome regions at 7q, 8p, 8q, and 17p in prostate cancer, we undertook a detailed normal value study. This normal-value study was necessary because the FISH analysis was performed using 5-μm sections from paraffin-embedded prostate specimens. In such specimens, nuclear truncation and nuclear overlap can cause false signal loss and false signal gain, respectively. For the normal-value study, each of the LSP/CEP dual-probe pairs was hybridized to 22 apparently benign prostate regions. Only normal epithelial cells were enumerated. For each normal region, the FISH data were tabulated and the FISH variables calculated as described in Materials and Methods. In benign epithelia, the mean LSP/CEP ratio for the 4 LSPs ranged from 0.98 to 1.04. The mean percentage of epithelial nuclei with −CEP and +CEP ranged from 21.8 to 25.8% and 1.26 to 1.61%, respectively. The mean percentage of epithelial nuclei with −LSP and +LSP ranged from 19.1 to 24.7% and from 0.9 to 1.3%, respectively.

Criteria for FISH Anomalies

On the basis of the normal value study and an inspection of the FISH signals distributions among the carcinoma foci, we developed the following conservative criteria for LSP and CEP copy number anomalies: 1) simple signal gain (+LSP or +CEP) required ≥10% epithelial nuclei with ≥3 signals for the probe studied, +LSP also required a mean LSP/CEP ratio of >0.86 and <1.30; 2) substantial additional increases (AI) of LSP copy number relative to centromere copy number required a mean LSP/CEP ratio of ≥1.30 and epithelial nuclei with ≥10% epithelial nuclei with ≥3 LSP signals; 3) simple signal loss (−LSP or −CEP) required ≥55% of epithelial nuclei with 0–1 signal for the probe studied; 4) relative loss (R-LSP) of LSP copy number required a mean LSP/CEP signal ratio ≤0.86.

Comparison of FISH Anomalies in Primary and Metastatic Carcinoma Foci

We performed detailed histologic mapping of all primary cancer foci and lymph node metastases for each patient. Examples are illustrated in Table 3. For example, Case 14 had four separate primary cancer foci with a Gleason score of 7 and four metastatic cancer foci, which involved 2 left obturator nodes, 1 left internal iliac node, and 1 right internal iliac node (Table 3). Figure 1 illustrates typical FISH results for one focus observed for this case. On the basis of our abnormal criteria, we defined all primary tumors in this case as having −8p22, +CEP8, +c-myc, +CEP7, +7q31, +CEP17, and −p53. Four lymph node metastases were classified as having the same anomalies as the primary tumors. Table 3 also illustrates another histologic map from a patient with Stage $T_{2a}N_2M_0$ prostate cancer (Case 36). This case had three primary tumors and three obturator node metastases. Both primary carcinoma foci were defined as having −8p22, +CEP8, and +c-myc, whereas three lymph node metastases were classified as having −8p22, +CEP8, +c-myc, +CEP7, +7q31, +CEP17, and −p53.

In 36 of the 37 patients with both primary and metastatic cancers available for study, FISH anomalies were observed in at least one focus of primary tumor and one focus of lymph node metastasis (the exception was Case 55; see Table 3). One or more similar anomalies were shared in paired primary and metastatic cancers in all of these 36 cases. For each of these 36 cases, the lymph node metastatic foci were located on the ipsilateral side of the patients, corresponding to the side of the prostate with the matched focus of cancer.

The overall incidence of signal copy number anomalies in Stage $T_{2-3}N_{1-3}M_0$ prostate cancer was 98% for the primary cancers and 99% for the lymph node metastases. In primary tumors, +CEP7, +7q31, −8p22, +CEP8, +c-myc, AI-c-myc, +CEP17, and −p53 were observed in 67%, 65%, 74%, 56%, 43%, 29%, and 31% of foci, respectively.

To further analyze the chromosome 8 anomalies in each cancer focus, we classified the copy number status of 8p, CEP8, and c-myc into 11 patterns (Table 4). Only six primary cancer foci and one metastatic cancer focus had no FISH anomalies for

TABLE 3. FISH Anomalies in Representative Cases with Stage $T_{2-3}N_{1-3}M_0$ Prostate Cancer

Patient	Lesion[a]	Location[b]	Gleason Score	FISH Anomalies[c]
Case 14	CA1	Right side	7	R−8p, +8c, +myc, +7c, +7q, +17c, −p53
	CA2	Right side	7	−8p, +8c, +myc, +7c, +7q, +17c, −p53
	CA3	Left side	7	R−8p, +8c, +myc, +7c, +7q, +17c, R−p53
	CA4	Left side	7	−8p, +8c, +myc, +7c, +7q, +17c, R−p53
	LN1	Left iliac		R−8p, +8c, +myc, +7c, +7q, +17c, −p53,
	LN2	Left obturator		−8p, +8c, +myc, +7c, +7q, +17c, −p53
	LN3	Left obturator		−8p, +8c, +myc, +7c, +7q, +17c, R−p53
	LN4	Right iliac		−8p, +8c, +myc, +7c, +7q, +17c, R−p53
Case 17	CA1	Left side	9	−8p, +8c, +myc, +7c, AI−7q, +17c, R−p53
	CA2-a	Right side	7	−8p, +7c, +7q, −p53
	CA2-b	Right side	7	−8p, −p53
	LN1	Right obturator		−8p, −p53
	LN2	Left obturator		−8p, +8c, AI-c-myc, +7c, +7q, +17c, R−p53
Case 23	CA1	Right side	7	−8p
	CA2	Left side	7	−8p
	LN1	Left obturator		−8p
	LN2	Right iliac		−8p, +7c, +7q
	LN3	Right iliac		−8p, +8c, +myc, +17c, +p53
	LN4	Right iliac		−8p, +8c, +myc
Case 36	CA1	Left side	5	−8p, +8c, +myc
	CA2	Right side	7	−8p, +8c, +myc
	CA3	Right side	7	−8p, +8c, +myc
	LN1	Right obturator		−8p, +8c, +myc, +7c, +7q, +17c, −p53
	LN2	Right obturator		−8p, +8c, +myc, +7c, +7q, +17c, −p53
	LN3	Right obturator		−8p, +8c, +myc, +7c, +7q, +17c, −p53
Case 38	CA1-a	Right side	7	−8p, −7c, −7q, +17c, −p53
	CA1-b	Right side	7	−8p, +8c, +myc, −7c, −7q
	CA2-a	Left side	7	−8p, +7c, +7q, +17c
	CA2-b	Left side	7	−8p, −7c, −7q, +17c, +p53
	LN1-a	Right iliac		−8p, −7c, −7q, −p53
	LN1-b	Right iliac		−8p, +8c, +myc, −7c, −7q, +17c, −p53
	LN2	Left pelvic		−8p, +8c, +myc, −7c, −7q
Case 55	Ca	Both sides	5	+7c, +7q
	LN	Right iliac		

p, p arm; q, q arm; c, centromere; AI, additional increase of copy number relative to the centromere; −a, −b, heterogeneity of FISH anomalies in the same cancer focus.

[a] CA, primary prostate cancer; CA1, dominant cancer focus; LN, prostate cancer lymph node metastasis.

[b] Indicating the location of primary cancer in the prostate and of metastasis in the lymph nodes.

[c] FISH anomalies, +, −, R−, indicating gain, loss, and relative loss of copy numbers for probes studied, respectively.

all three loci of chromosome 8. The most common patterns included the following: loss-normal-normal (8p-CEP8-c-myc), which was observed in 22 (18%) primary tumors and 14 (12%) metastatic foci ($P = .45$); loss-gain-gain was identified in 45 (38%) primary tumors and 63 (55%) metastatic foci ($P = .25$). Loss-Gain-AI was identified in 18 (15%) primary tumors and 14 (12%) metastatic foci ($P = .81$).

The most common pattern of CEP7 and 7q31 copy number status was gain-gain (CEP7–7q31), which was observed in 72 (67%) primary tumors and 77 (75%) metastatic foci ($P = .52$). AI-7q31 was only observed in five primary cancer foci and four lymph node metastases.

The most common patterns of CEP17 and p53 copy number status were gain-gain (CEP17-p53), which was observed in 18 (16%) primary tumors and 32 (29%) metastatic foci ($P = .19$), and gain-loss, which was observed in 27 (24%) primary tumors and 26 (24%) metastatic foci ($P = .99$).

We next examined the incidence of specific combined patterns of 8p, c-myc, 7q31, and p53 anomalies (Table 5). We identified 19 total patterns, of which 3 were the most common: Pattern I, with −8p, +c-myc or AI-c-myc, and +7q, which was observed in 23 (21%) primary tumors and 28 (27%) metastatic foci ($P = .53$); Pattern II, with −8p, +c-myc or AI-c-myc, +7q, and −p53, which was observed in 12 (11%) primary tumors and 13 (13%) metastatic foci ($P = .75$); and Pattern III, with −8p, +c-myc or AI-c-myc, +7q, and +p53, which was observed in 6 (6%) primary tumors and 22 (21%) metastatic foci ($P = .08$). Only 13 (12%) primary cancer foci and 8 (8%) lymph node metastases showed FISH anomalies without 8p involvement.

Intraglandular and Intrafocus Heterogeneity of Genetic Changes in Primary and Metastatic Cancer Foci

There was considerable variability in the frequency of FISH anomalies among different primary tumors in a single prostate. In 61% of cases (25/41) with multiple primary cancer foci, anomalies were observed in one or more cancer foci but not in

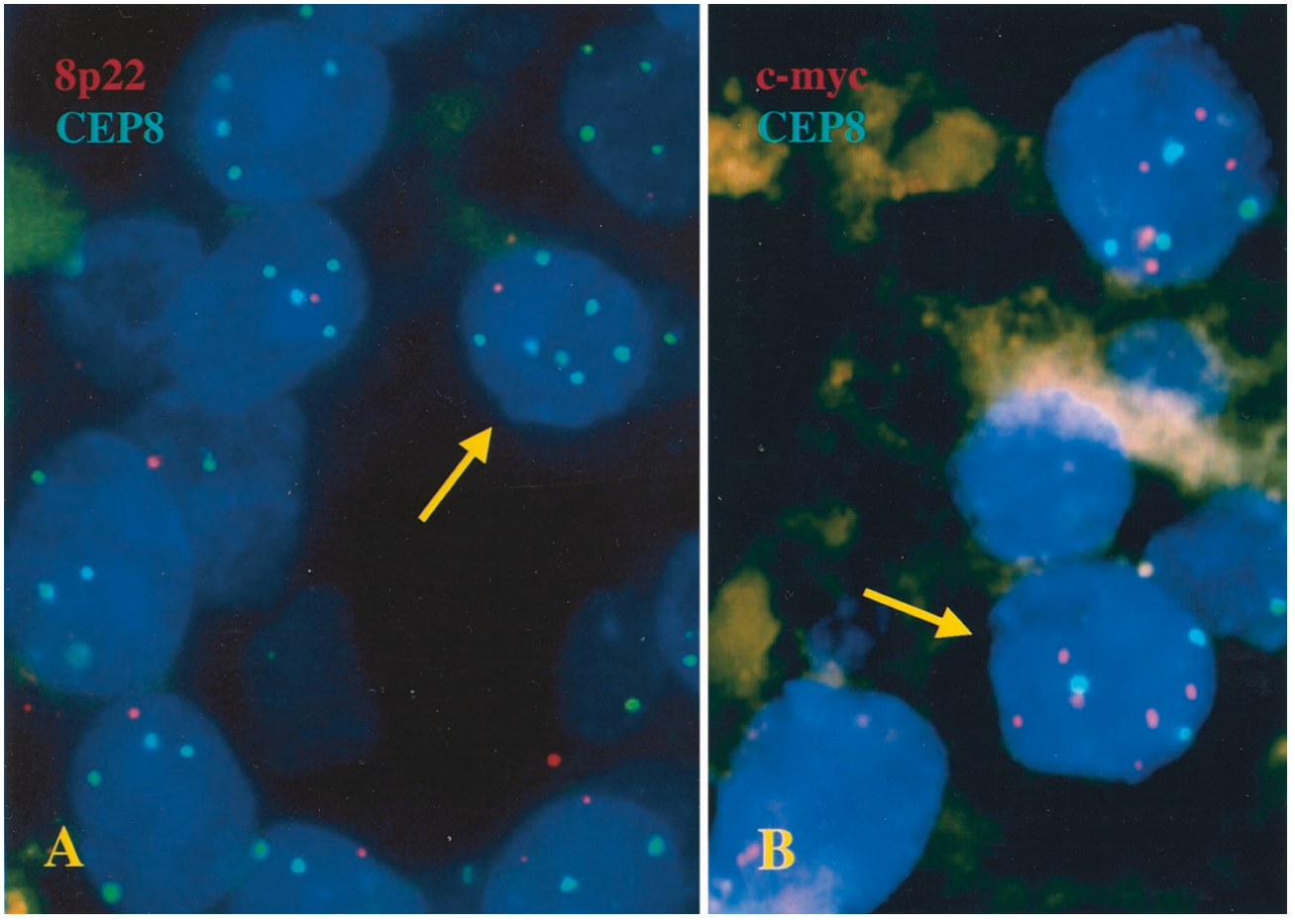

FIGURE 1. Dual-probe FISH with a centromere probe for chromosome 8 (***CEP8, green***), and region-specific probes for LPL and c-myc (***orange***), in representative fields of Case 14 (Stage $T_{3b}N_2M_0$). Nuclei are counterstained with DAPI. **A**, a cancer epithelial nucleus has seven signals for chromosome 8 and one signal for LPL (***arrow***). **B**, a cancer cell nucleus has three signals for chromosome 8 and six signals for the c-myc oncogene (***arrow***).

TABLE 4. Summarized Patterns of Genetic Changes on Chromosome 8 in Stage $T_{2-3}N_{1-3}M_0$ Prostate Cancer

Patterns	8p[a]	CEP8	c-myc	Number of Cancer Foci (%)		*P* Value[b]	Number of Patients in Each Group (%)		*P* Value[c]
				Primary	Metastasis		Case	Control	
1	Normal	Normal	Normal	6 (5)	1 (1)	.048	1 (4)	1 (4)	1.00
2	Loss	Normal	Normal	22 (18)	14 (12)	.45	2 (8)	4 (15)	.42
3	Loss	Loss	Normal	3 (3)	2 (2)	.57	0 (0)	0 (0)	—
4	Loss	Gain	Gain	45 (38)	63 (55)	.25	8 (31)	11 (42)	.37
5	Gain	Gain	Gain	6 (5)	1 (1)	.07	1 (4)	3 (12)	.34
6	Loss	Loss	Loss	2 (2)	0 (0)	—	0 (0)	1 (4)	.99
7	Normal	Normal	AI	4 (3)	1 (1)	.07	0 (0)	0 (0)	—
8	Loss	Normal	AI	7 (6)	8 (7)	.83	6 (23)	0 (0)	.10
9	Loss	Loss	AI	5 (4)	3 (3)	.81	1 (4)	2 (8)	.57
10	Loss	Gain	AI	18 (15)	14 (12)	.81	7 (26)	3 (12)	.21
11	Gain	Gain	AI	1 (1)	7 (6)	.08	0 (0)	0 (0)	—
Total				119 (100)	114 (100)		26 (100)	26 (100)	

[a] Loss of 8p in this table includes −8p and relative −8p (see definition in "Results").
[b] Comparing primary tumor versus lymph node metastasis using GEE models.
[c] Comparing case group versus control group using conditional logistic regression.

other cancer foci in a single prostate, indicating significant intraglandular cancer genetic heterogeneity (see Cases 17 and 38 in Table 3). However, in only 28% of patients (7/25) with multiple positive nodes, some anomalies were observed in one or more nodes but not in other lymph node metastases from the same case (see Cases 17 and 23 in Table 3). Usually, these positive nodes with different anomalies were located on opposite sides of the body (90% of cases).

TABLE 5. Summarized Patterns of Genetic Changes on Chromosomes 7, 8, and 17 in Stage $T_{2-3}N_{1-3}M_0$ Prostate Cancer

Patterns	Combined Genetic Changes	Number of Cancer Foci (%)		*P* Value[a]	Number of Patients in Each Group (%)		*P* Value[b]
		Primary	Metastasis		Case	Control	
I	−8p, +c-myc or AI-myc, +7q	23 (21)	28 (27)	.54	4 (18)	7 (32)	.27
II	−8p, +c-myc or AI-myc, +7q, −p53	12 (11)	13 (13)	.75	6 (27)	2 (9)	.18
III	−8p, +c-myc or AI-myc, +7q, +p53	6 (6)	22 (21)	.08	0 (0)	3 (14)	.99
IV	Other 16 patterns	65 (60)	40 (39)	.06	12 (55)	10 (45)	.53
Total[c]		108 (100)	103 (100)		22 (100)	22 (100)	

[a] Comparing primary tumor versus lymph node metastasis using GEE models.
[b] Comparing case group versus control group using conditional logistic regression.
[c] Because there was not enough tissue, FISH analysis of Chromosome 7 was not performed on all foci and cases.

Intrafocus heterogeneity (heterogeneity within a single cancer focus) of FISH anomalies was frequent in primary carcinoma foci. We studied 17 dominant primary carcinoma foci and found intrafocus heterogeneity in 12 foci (71%). For example, in Case 38 (Table 3), the Cancer Focus 1 had a total Gleason score of 7; tumor cells with Gleason Primary Pattern 4 (CA1-a) showed −8p, -CEP7, −7q31, +CEP17, and +p53, whereas tumor cells with Secondary Pattern 3 (CA1-b) showed −8p, +CEP8, +c-myc, −CEP7, and −7q31. However, very little intrafocus heterogeneity was observed in the lymph node metastases, with the exception of Case 38, which showed more genetic changes in the cancer cells located in the central area (LN1-b) of metastasis than in the surrounding area (LN1-a; data not shown and Table 3).

Comparison of FISH Anomalies in Primary Tumors between Case and Control Groups

Figure 2 summarizes the genetic changes in primary tumors between matched case and control groups. If one case had multiple cancer foci, the cancer focus with the most genetic changes was chosen to represent the case. All prostates (100%) contained alterations for at least one marker studied. As can be seen in Figure 2, the frequency of AI-c-myc in the case group was slightly higher than in the control group (54% *versus* 23%, $P = .057$, odds ratio = 3.0, 95% confidence interval = 1.0–9.3). The frequency of −p53 in case group was higher than in control group (46% *versus* 15%, $P = .04$, odds ratio = 5.0, 95% confidence interval = 1.1–22.8). Tables 4 and 5 also compare the incidence of selected genetic alteration patterns in case and control groups. No significant differences were observed.

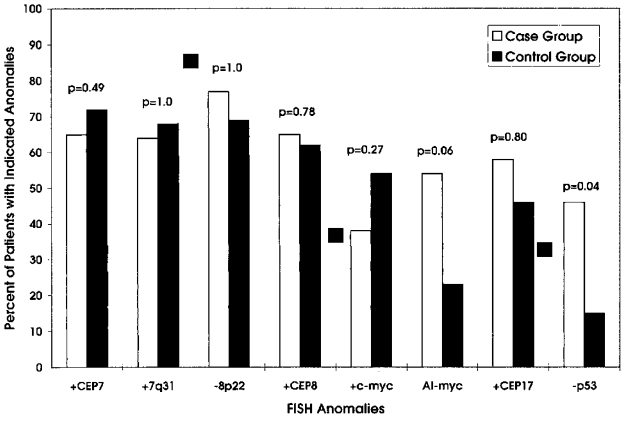

FIGURE 2. Comparison of the most common FISH anomalies between case and control groups in Stage $T_{2-3}N_{1-3}M_0$ primary prostate cancers. *P* values have been calculated for all markers, comparing 26 patients in the case group with 26 patients in the control group for LPL, CEP8, and c-myc. Because there was not enough tissue for FISH analysis, 22 patients in the case group and 22 patients in the control group were evaluated for CEP7 and 7q31, and 25 patients in the case group and 25 patients in the control group were evaluated for CEP17 and p53.

The Relationship of FISH Anomalies with Pathologic Tumor Characteristics

There was a positive relationship of the Gleason score with AI-c-myc ($P = .003$) in primary carcinoma. The incidence of AI-c-myc was observed in 18% for Gleason score of <7, 24% for score of 7, and 48% for score of >7, respectively. No significant relationship with Gleason score was observed for the other FISH anomalies detected in this study. In addition, there was no correlation between tumor volume or multifocality with the number or type of genetic alterations (data not shown).

DISCUSSION

We performed a comprehensive retrospective case-control analysis evaluating the prognostic value of specific chromosome and gene copy number anomalies detected by FISH in Stage $T_{2-3}N_{1-3}M_0$ prostate cancer. This study also contains the largest set of comparative FISH data compiled to date in a series of matched multiple prostatic primary tumors and multiple lymph nodal metastases.

We found that FISH anomalies were very common in advanced-stage cancer. We also found that AI-c-myc was associated with higher cancer Gleason score

and that the incidence of AI-c-myc and −p53 in Stage $T_{2-3}N_{1-3}M_0$ prostate cancer was higher in the poor prognosis group than in the good prognosis group. Our results indicate that multiple chromosomal and genetic alterations are involved in carcinogenesis of the prostate.

Like the case with other molecular genetic studies (7–11), we observed frequent loss of 8p in prostate cancer. For example, using the laser microdissection technique, Emmert-Buck *et al.* (10) identified LPL loss in 91% of prostate cancer and in 63% of its precursor, prostatic intraepithelial neoplasia (PIN). In addition, Bostwick *et al.* (24) found LPL loss in 50% of prostate cancer and in 41% of PIN. In this study, we found that >80% of Stage $T_{2-3}N_{1-3}M_0$ prostate cancer had loss of the LPL locus, but this loss was not associated with cancer progression. Together with previous data, this study suggests that −8p is an early event in carcinogenesis of the prostate.

The c-myc gene overrepresentation also appears to contribute to prostatic carcinogenesis. c-myc, present at 8q, was one of the first cellular oncogene cloned (25, 26). However, much has been recently learned about the biology of the gene itself (27). It has been reported that overexpression of c-myc in model systems increases cellular proliferation rates, decreases apoptosis, and in some systems, increases tumorigenicity (27). The c-myc gene has been found to be overexpressed and/or amplified in prostate cancer (12–15, 28, 29). For example, Visakorpi *et al.* (14) found +8q more frequently in locally recurrent cancer than in the primary tumor and found amplification of 8q DNA sequences in 75% of lymph node metastases. Cher *et al.* (28) identified a high frequency (85%) of +8q in prostate cancer metastases. We have previously reported frequent amplification of the c-myc gene in metastatic prostate cancer (21%) (12) and have recently shown that AI-c-myc is associated with a poorer overall survival and progression-free survival in men with Stage $pT_3N_0M_0$ prostate cancer (15). In this study, we found that patients with AI-c-myc had a trend toward worse prognosis than those without AI-c-myc ($P = .06$). AI-c-myc was also strongly associated with higher Gleason score. Interestingly, Alers *et al.* (29) observed overrepresentation of 8q sequences in prostate cancer bone metastases. These data suggest that c-myc gene amplification and overexpression alone and/or with other gene(s) mapped to 8q may be a late event in carcinogenesis of the prostate and play an important role in prostate cancer progression and evolution. Thus, overexpression of c-myc may be a potential marker of poor prognosis in prostate cancer.

Cytogenetic and molecular genetic studies have demonstrated that gains of chromosome 7 and 7 q-arm alterations were frequent in prostate cancer (30, 31). We previously reported that allelic imbalance of 7q31 is common in prostate cancer and is associated with higher tumor grade and advanced pathologic stage (19). We also observed that allelic imbalance of 7q31 is strongly associated with Stage $T_3N_0M_0$ prostate cancer progression and patient death (20). Recently, using closely linked locus-specific probes, we confirmed that 7q31 is frequently altered in prostate cancer (32). We also found that apparent simple gain of the chromosome 7 q-arm, including the centromere and additional gain of the 7 q-arm relative to the centromere, were more common than simple loss of the 7 q-arm or deletion of 7q31 (32). Using comparative genomic hybridization (CGH), Visakorpi *et al.* (14) found that gain of chromosome 7 was much more common in recurrent prostate cancer than in primary prostate cancer. By CGH, Cher *et al.* (28) also detected frequent gain of the 7 q-arm in metastatic and androgen-independent prostate cancer. In this study, we found that overrepresentation of the 7 q-arm was very common in Stage $T_{2-3}N_{1-3}M_0$ prostate cancer. The accumulative findings suggest that overrepresentation of the 7 q-arm, and possibly the 7q31 region, is important for prostate cancer progression.

The p53 gene is one of the most intensively studied tumor suppressor genes and was previously shown to be mutated in 20–45% of metastatic prostate cancers (33–35). In addition, p53 mutation has been reported in 8 (50%) of 16 prostate cancer bone marrow metastases (4). Cher *et al.* (28) reported that 50% of prostate cancer lymph node metastases and 65% of androgen-independent tumors had a −17p. Brooks *et al.* (18) reported that allelic loss of chromosome 17p appeared to be highly correlated with prostate cancer recurrence. Thompson *et al.* (34–36) used a mouse prostate reconstitution model system in strains of p53 knockout mice to test the role of p53 in the prostate cancer metastasis. In this model, ras and c-myc–initiated carcinomas in heterozygous p53 mice invariably showed metastases and systematic progression with complete loss, partial deletion, or loss of expression of the wild-type p53 allele (34–36). In this study, we found that −p53 was associated with survival. Together, these data support the view that loss of normal p53 function is associated with prostate cancer progression. It also appears to be an alteration that occurs most commonly in late stages of prostate cancers and may be a marker of survival in Stage $T_{2-3}N_{1-3}M_0$ prostate cancer.

We evaluated patterns of FISH anomalies to develop a model of the preferred genetic alterations sequence in prostate cancer. The most common patterns were as follows: Pattern I, with −8p, +c-myc or AI-c-myc, and +7q; Pattern II, with −8p,

+c-myc or AI-c-myc, +7q, and −p53; and Pattern III, with −8p, +c-myc or AI-c-myc, +7q, and +p53. We found that only 12% of primary cancer foci and 8% of lymph node metastases showed FISH anomalies without 8p involvement, indicating that +c-myc or AI-c-myc, +7q, and −p53 rarely occurred in the absence of −8p. We also observed that the incidence of the genetic change pattern of −8p, +c-myc or AI-c-myc, +7q, and +p53 were slightly higher in lymph node metastases than in primary tumors ($P = .08$). These findings suggest that multiple genetic changes are required for the tumor to metastasize to local lymph nodes and/or that metastatic lesions are genetically unstable.

This work extends previous findings regarding the relationship of the genetic changes in lymph node metastases with those in prostatic carcinoma. For example, Sakr *et al.* (37) reported that some lymph node metastases did not share genetic alterations with the primary tumor, but this result may have been caused by incomplete sampling of prostate tumor foci. Further analyses indicated that metastases were not necessarily derived from the most abundant clone in the primary tumor (12, 22). In this study, we carefully evaluated several chromosomal regions by FISH in all multiple primary cancer foci and corresponding lymph node metastases. We found that one or more primary tumors usually shared the same FISH alterations as those in corresponding ipsilateral multiple lymph node metastases, suggesting that this tumor focus gave rise to the metastatic lesions. However, seven patients showed different genetic changes among the multiple metastases in a single case. Usually, these positive nodes with different anomalies were located on opposite sides of the body, indicating that perhaps more contralateral cancer foci give rise to independent metastases and/or that metastatic lesions are genetically unstable (38). In addition, we found that the metastatic cancer cells within the same lymph node usually have homogeneous histologic characteristics and chromosomal anomalies (data not shown on Table 3). This suggests that a single metastasized cancer cell clone with multiple genetic changes expands quickly in a lymph node (39–41).

Prostate cancer is usually multifocal (22). We previously reported frequent intraglandular and intratumoral genetic heterogeneity of prostate cancer in whole mounted prostates (12, 22). In this study, the dominant (largest) focus of carcinoma usually showed more genetic changes than other smaller foci (Table 3). However, FISH analysis showed that some small low-grade tumor foci had a high frequency of genetic changes by FISH, whereas concurrent dominant high-grade tumor foci were normal, which indicates that small cancers can have significant alterations (12, 22). Using PCR, ISH, and DNA ploidy techniques, similar intraglandular and intratumoral heterogeneity has been reported (12, 24, 40, 41). Thus, the size of a cancer focus and its degree of histologic dedifferentiation may not reflect the extent of its genetic derangement (12, 22, 24, 40, 41). These results also indicate that without broad and systematic scrutiny of all neoplastic foci in a prostate, significant genetic changes will not be detected (12). Because this study contains only a small number of patients, an extended study with a large group of Stage $T_{2-3}N_{1-3}M_0$ patients will be useful to further confirm our preliminary findings.

In conclusion, this study shows that FISH anomalies are very common in both primary tumors and lymph node metastases of Stage $T_{2-3}N_{1-3}M_0$ prostate cancer. It also shows that AI-c-myc is associated with higher cancer Gleason score and that AI-c-myc and −p53 are associated with prostate cancer progression; they are potential markers of survival in Stage $T_{2-3}N_{1-3}M_0$ prostate cancer. Finally, it shows that lymph node metastases usually have similar or additional genetic changes compared with primary tumors and that multiple lymph node metastases usually have similar genetic changes.

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