[CANCER RESEARCH 54, 3273–3277, June 15, 1994]

Allelic Loss in Locally Metastatic, Multisampled Prostate Cancer[1]

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ABSTRACT

In order to determine whether retention or loss of potential tumor suppressor loci that map to 8p, 10q, or 16q reflect genetic relationships among prostatic intraepithelial neoplasias (PINs), multicentric primary prostatic cancers, and regional lymph node metastases or are associated with the metastatic phenotype, we analyzed 19 cases of locally metastatic prostate carcinoma (stage D1) utilizing polymerase chain reaction techniques. In each case, tissue samples from metastatic tumor, the (dominant) primary tumor, and nonneoplastic prostatic tissue were examined. In selected cases, allelic loss in additional tumor foci, separate from the dominant tumor nodule, and areas of PIN were examined. Allelic loss of sequences on 8p, 10q, and 16q were observed in 20–29% of PINs, 18–42% of primary tumors, and 8–25% of metastatic tumors. Discrepancies in sequence dosage between histological components were most pronounced for 8p sequences, especially between the dominant tumor nodule and metastatic deposits in cases in which ≥3 separate tumor foci/gland were identified. These results suggest that putative premalignant lesions, moderately or poorly differentiated, geographically separate primary tumor foci, and metastases within morphologically "complex" prostates (those with ≥3 foci/gland) are likely to be more discordant for sequence dosage at 8p than those within "simpler" glands (<3 foci/gland). Also, our results suggest that lymph node metastases may be genetically related to either the dominant or additional primary tumor foci in more complex prostates and that accumulation of genetic aberration may differ in primary and metastatic lesions.

INTRODUCTION

Carcinoma of the prostate, the most frequently diagnosed cancer in American males, continues to show a steady increase in the annual incidence of newly diagnosed cases (1). This tumor is characterized by a remarkably variable, often prolonged natural history (2). A significant, but unknown, proportion of newly diagnosed prostate cancers, in particular those of small volume that are well differentiated, are assumed to be clinically indolent and may not require aggressive treatment. Our ability to predict the biological course of individual tumors, however, is extremely limited, emphasizing the need to identify objective biological "markers" of malignant potential in each case. The development of lymph node metastases from a primary prostatic carcinoma is associated with significantly higher mortality in comparison with localized cancer (3). The tumor subpopulation achieving successful implantation in the pelvic lymph nodes is presumed to be associated with markers linked to, and therefore predictive of, the metastatic phenotype. Candidate genetic markers include deleted sequence domains within or near putative tumor suppressor genes. Recent studies have identified chromosomal regions on 8p, 10q, and 16q that are frequently deleted in human prostate cancers (4–7).

In addition to the dominant tumor nodule, most radical prostatectomy specimens from patients with clinically localized carcinoma of the prostate contain one or more anatomically separate, smaller tumor foci, often of different histological grades. In order to examine the genetic relationships between multiple tumor nodules and metastatic deposits in the same patient, we have analyzed microdissected samples from both the primary tumor and the lymph node metastases, utilizing PCR techniques. We sought to (*a*) investigate how retention or deletion of alleles of putative tumor suppressor loci at 8p, 10q, or 16q are distributed between PIN,[4] small geographically separate well-differentiated tumor foci, dominant (usually less differentiated) tumor nodules, and metastases and (*b*) determine whether allelic losses at 8p, 10q, or 16q are associated with the metastatic phenotype and constitute genetic markers of progression. If we assume that these markers characterize the more aggressive tumors, then identifying them in the smaller, nondominant diagnosed cancers may be helpful in predicting progression and metastatic potential of tumors in individual patients.

MATERIALS AND METHODS

Patient Selection, Pathological Evaluation, and Tissue Microdissection. Twenty-six consecutive patients with pathologically established stage D1 prostatic carcinoma (metastatic carcinoma to iliac/obturator lymph nodes) were identified from the recent surgical pathology files of Harper Hospital (between 1990–1992). In 7 patients, the lymph node metastases were minute microscopic foci insufficient for serial sectioning. A total of 19 patients with adequate tumor volume in regional lymph nodes were evaluated.

The radical prostatectomy specimen and dissected lymph nodes are processed according to a uniform protocol[5] (7) in which the specimen is delivered fresh to the tissue laboratory, inked, and sliced at 3- to 4-mm intervals perpendicular to the posterior surface of the gland. The resulting slices are sampled for special studies from the grossly identified tumorous and benign areas. The distribution of disease and the exact source of each sample is indicated clearly on a preliminary diagram. Subsequently, the entire gland is submitted for microscopic evaluation, and a final diagram of the prostatic diseases is generated documenting anatomic distribution and multifocality when present (Fig. 1, *left*). The tumor areas are mapped in red ink indicating the geographic and zonal distribution of the neoplasm(s) and the states of the surgical margins, seminal vesicles, and extraprostatic tissue. The source of the special study samples is also indicated on the final diagram, allowing for a highly accurate microscopic characterization of each mirror image sample [proportion of tumor present, histological grade(s), proportions of benign epithelium, stroma, premalignant lesions, etc.]. It is feasible to establish prostate cancer multicentricity, document its zonal distribution, and assess the relative volume of each tumor nodule by reconstructing the step-sectioned, entirely submitted prostate gland[5] (6, 7).

In all 19 cases, the following histological components were analyzed: metastatic tumor deposits from one or more lymph nodes, samples of the dominant (largest, primary) tumor nodule in the prostate, and normal (either benign or benign hyperplastic) prostatic epithelium as control. In 10 cases, one additional separate and smaller primary tumor focus was examined. In 9 cases, high-grade PIN (not admixed with carcinoma or with more than 50% of intervening benign tissue) was examined. The tumors were graded according

Received 12/28/93; accepted 4/11/94.

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[1] This work was supported, in part, by a grant from Merck, Sharpe and Dohme and funds from the Departments of Pathology and Urology, Wayne State University School of Medicine, Detroit, MI.

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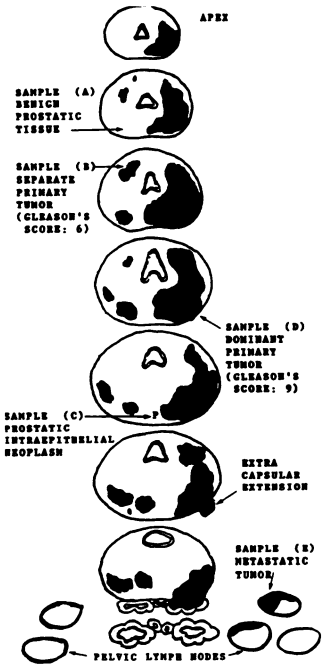
[4] The abbreviations used are: PIN, prostatic intraepithelial neoplasia; H&E, hematoxylin and eosin; PCR, polymerase chain reaction.

[5] W. A. Sakr, D. J. Grignon, D. W. Visscher, J. A. Macoska, J. E. Pontes, J. E. Montie, S. R. Wolman, and J. D. Crissman. Evaluating the radical prostatectomy specimen: a protocol for establishing prognostic parameters and defining genetic abnormalities, manuscript submitted.



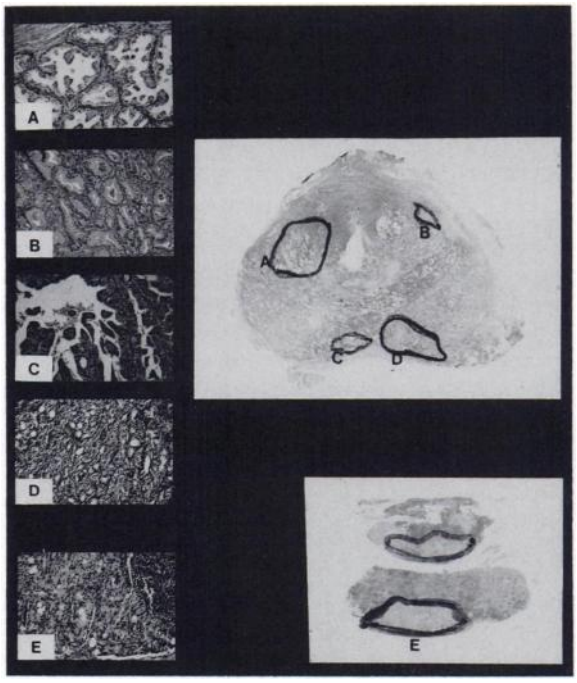


Fig. 1. *Left,* anatomic distribution of prostatic carcinoma with illustration of the source of the tissue samples obtained from the dominant and additional primary tumor foci, premalignant lesions, benign prostatic tissue, and metastatic tumor deposits. *Right,* microdissection of the histological counterpart of benign prostatic tissue (*A*), moderately differentiated transition zone carcinoma (*B*), high-grade prostatic intraepithelial neoplasia (*C*), poorly differentiated peripheral zone carcinoma (*D*), and metastatic adenocarcinoma in pelvic lymph nodes (*E*).

to the method of Gleason (8). Only tumors with at least 70% malignant cells within the circumscribed areas were submitted for molecular analysis. Criteria described by McNeal and Bostwick (9) were applied to classify PIN lesions.

These histological areas were circled on the H&E-stained slide(s) (Fig. 1, *right*). Three 4-μm-thick sections were obtained from the corresponding paraffin-embedded tissue blocks and applied to silanized slides without coverslips. The middle section was stained with H&E to ensure the preservation of histological components. Guided by the marked H&E slides, areas with cancer, PIN, and benign tissue were circumscribed on the blank sections for molecular analysis.

DNA Purification and PCR Analysis. DNA was extracted from the deparaffinized, microdissected tissue samples as described previously (6). PCR analysis targeted sequences containing highly polymorphic microsatellite repeat motifs at loci of interest on chromosomal regions 8p22 (*LPL* locus, LPL-5′-CA and LPL-5′-GT primers), 10q11.2-qter (*D10S91* locus), 12pter-p12 (*F8VWF* locus), and 16q22.1 (*D16S289* locus). Oligonucleotide primers to sequences amplified at 8p, 10q, and 16q have been described elsewhere (7, 10). Oligonucleotide primers to sequences at the *F8VWF* locus (12pter → p12) (used as a dosage control) were as follows: 5′-ATTTCCACCCACTTCTG-3′ (sense) and 5′-ATGGTGTTGAGAATTAGGC-3′ (antisense).[6] PCR reactions and autoradiography were performed as described previously (7). Quantitation of autoradiographic signal was accomplished by scanning laser densitometry and comparison of the ratio of allelic signal intensities with normal (as control) and other tissues. Allelic loss was scored when the ratio of allelic signal intensities in tumor or PIN tissue was ≤50% of the ratio obtained for normal tissue from the same patient.

Statistical Analysis. Statistical analysis was performed utilizing a χ^2 (Fisher exact) test, with P values <0.05 considered statistically significant.

RESULTS

Morphology. The dominant primary tumors analyzed were extensive and moderate to poorly differentiated: the mean percentage of glandular involvement by tumor was 40% (range, 10–70%). The mean Gleason's score was 8 (range, 6–9). Seventeen of the 19 tumors (89%) showed established extraprostatic extension, and 15 (79%) extended into the seminal vesicles on one or both sides.

The detailed mapping of tumor distribution allowed for a reasonably accurate assessment of multicentricity and extent of glandular involvement by tumor in 16 of 19 prostates (Fig. 1, *left*; Table 1). Thirteen of 16 (81%) were multicentric as defined by our ability to distinguish 2 or more anatomically separate tumor foci. Twelve prostates harbored distinct tumor foci in both the peripheral and transition zones. Four had peripheral zone tumors only. Our ability to establish multicentricity was compromised in cases with large tumor volume and extensive glandular involvement. Therefore, large cancers that replaced most of the prostate, often with both peripheral and transition zone distribution (peripheral zone cancers extending into the transition zone, 3 cases), were considered one large tumor. The number of tumor foci ranged from 1–8 (mean, 3 foci/gland). Accordingly, we defined prostates containing ≥3 separate tumor foci as morphologically "complex," and those with <3 foci were defined as "simple." Nine of the 19 prostates (47%) examined contained distinct areas of high grade PIN (Table 1).

In 17 cases (89%), the side of the lymph node metastasis corresponded to that of the dominant tumor nodule in the gland. In the remaining 2 cases (cases 8 and 14), the pelvic/obturator lymph nodes harboring metastatic tumor were contralateral to the dominant primary tumor (Table 1).

Molecular Analysis. Data concerning retention or deletion of sequences at 8p, 10q, and 16q in different histological components from each case is presented in detail in Table 2. Overall allelic loss frequencies in normal prostatic tissue, PIN, and primary and metastatic tumors are summarized in Table 3. Allelic losses were observed for 8p (29%), 10q (18%), and 16q (42%) in primary tumor foci and for 8p (25%), 10q (12%), and 16q (8%), in metastatic tumors. Although a trend toward increased frequency of allelic loss for 8p, 10q, and 16q was observed with increasing tumor grade, these correlations were not statistically significant. PIN lesions demonstrated allelic loss frequencies similar to those of primary tumors. Allelic loss was not observed at any locus in normal or benign hyperplastic tissue. Allelic loss at 12p was not observed for any specimen. Gel autoradiographs depicting allelic loss of 10q sequences in a PIN specimen and of 16q sequences in a tumor specimen from case 14 are shown in Fig. 2, and gel autoradiographs depicting allelic loss of 8p sequences in lymph node metastasis, tumor, and PIN tissue from case #18 are shown in Fig. 3.

[6] J. L. Weber. Twenty-five new CA repeats, personal communication to GDB/OMIM; GDB identification 36129, 1992.

Table 1 *Morphological characteristics of the 19 cases analyzed*

Case	No. of separate tumor foci			Gleason's score of foci sampled		PIN (Y/N[a])	% glandular involvement	Dom tum nod same side as +LN
	PZ	TZ	Total	Dom	Adt,Sep			
1	1	1	2	PZ (8)		N	70	Yes
2	3	0	3	PZ (9)	TZ (4)	N	70	Yes
3	2	1	3	PZ (7)	TZ (6)	N	25	Yes
4	3	2	5	PZ (7)	TZ (5)	Y	40	Yes
5	1	2	3	PZ (9)	TZ (7)	N	60	Yes
6	5	3	8	PZ (9)		N	70	Yes
7	3	1	4	PZ (8)	TZ (6)	Y	40	Yes
8	4	3	7	PZ(10)	TZ (6)	Y	50	No
9	1	1	2	PZ (9)	TZ (6)	N	70	Yes
10	1	1	2	PZ (9)	PZ (8)	N	50	Yes
11	3	2	5	PZ (8)	TZ (5)	Y	35	Yes
12	2	1	3	PZ (8)	PZ (8)	Y	50	Yes
13	1	0	1	PZ(10)		N	10	Yes
14	1	0	1	PZ (9)		Y	20	No
15	1	1	2	PZ (7)		Y	25	Yes
16	1	0	1	PZ (7)		N	70	Yes
17			[b]	PZ (9)		Y	[b]	Yes
18			[b]	PZ (8)		Y	[b]	Yes
19			[b]	PZ (8)		N	[b]	Yes

[a] Y, yes; N, no; PZ, peripheral zone; TZ, transition zone; Dom, dominant; Adt, additional; Sep, separate; Dom tum nod same side as +LN, dominant tumor nodule at the same side of the positive pelvic lymph node.
[b] Could not be determined.

Comparison of any two histological components (normal tissue, dominant tumor nodule, additional tumor focus, PIN, or lymph node metastasis) revealed concordant or discordant sequence dosages at given loci. For example, comparison of the dominant tumor focus and lymph node metastasis for 8p loss in case 4 reveals retention of 8p sequences in both components. Comparison of the dominant tumor nodule and lymph node metastasis for 8p loss in case 18 reveals deletion of 8p sequences in both. Therefore, the dominant tumor nodule and metastasis from cases 4 and 18 are concordant with respect to 8p (in case 4, both retained 8p sequences, and in case 18, both are deleted for 8p sequences). Conversely, comparison of two histological components from a given case may show that they are discordant for sequence dosage. For example, the dominant tumor nodule and a second additional focus from case 7 differ in that the dominant tumor nodule is deleted for sequences on 8p that the other focus has retained. Therefore, the dominant nodule and additional focus from case 7 are discordant at 8p.

Examples of concordance and discordance between histological components for sequence dosage at 8p, 10q, and 16q may be deduced from the data in Table 2. Comparison of allelic loss patterns in simple or complex prostates shows that differences are observed for retention or loss of 8p but not 10q or 16q sequences between histological components (Table 4). For example, 8p loss in dominant tumor foci and metastases was concordant in 5 of 5 (100%) cases with <3 foci/glands but in only 3 of 6 (50%) cases with >3 foci/glands. Conversely, 10q and 16q allelic losses were concordant in 80 and 67% of prostates with <3 foci/glands and in 78 and 86% of prostates with >3 foci/glands. Comparisons between other histological components within more complex prostates shows that they more often differed in 8p allelic loss patterns than in 10q or 16q allelic loss patterns. For example, dominant and additional tumor foci were concordant for 8p allelic loss in only 3 of 5 (60%) cases but were concordant for 10q or 16q allelic losses in 5 of 7 (71%) and 6 of 6 (100%) cases, respectively. Although not statistically significant, these trends suggests that

Table 2 *Retention or deletion of alleles at 8p, 10q, or 16q in histological components from multisampled prostates*

Case	No.of foci		8p chromosomal region					10q chromosomal region					16q chromosomal region				
			Tissue type					Tissue type					Tissue type				
	T[a]	S	N	DF	AF	PIN	LN	N	DF	AF	PIN	LN	N	DF	AF	PIN	LN
1	1	1	R	R	NA	NA	R	R	L	NA	NA	R	R	R	NA	NA	R
2	3	2	R	R	R	NA	L	R	R	R	NA	L	R	L	L	NA	L
3	3	2	NI	NI	NI	NA	NI	R	R	R	NA	R	R	R	R	NA	R
4	5	2+P	R	R	R	R	R	R	R	R	R	R	NI	NI	NI	NI	NI
5	3	2	NI	NI	NI	NA	NI	R	R	L	NA	R	R	R	R	NA	R
6	8	1	R	R	NA	NA	R	R	R	NA	NA	R	NI	NI	NA	NA	NI
7	4	2+P	R	L	R	R	ND	R	R	R	R	R	R	R	R	R	R
8	7	2+P	R	R	R	R	L	R	L	R	R	R	R	L	R	R	R
9	2	2	NI	NI	NI	NA	NI	R	R	R	NA	R	R	ND	L	NA	R
10	2	2	NI	NI	NI	NA	NI	R	R	R	NA	R	R	R	R	NA	R
11	5	2+P	R	R	L	ND	ND	R	R	R	R	R	R	R	R	L	R
12	3	2+P	R	R	R	L	R	R	R	R	L	R	R	R	ND	R	R
13	1	1	R	R	NA	NA	R	R	R	NA	NA	R	ND	ND	NA	NA	ND
14	1	1+P	R	R	NA	R	R	R	R	NA	L	R	R	L	NA	R	R
15	2	1+P	R	R	NA	R	R	ND	ND	NA	ND	ND	ND	ND	NA	ND	ND
16	1	1	R	R	NA	NA	R	ND	ND	NA	NA	ND	ND	ND	NA	NA	ND
17[b]	ND	1+P	NI	NI	NA	NI	NI	R	R	NA	R	R	NI	NI	NA	NI	NI
18[b]	ND	1+P	R	L	NA	L	L	R	R	NA	R	R	NI	NI	NA	NI	NI
19[b]	ND	1	R	L	NA	NA	R	R	ND	NA	NA	L	R	L	NA	NA	R

[a] T, total number of foci in prostate gland; S, number of foci sampled in prostate gland (this number always includes the dominant tumor nodule; P, PIN focus).
Tissue type: N, normal prostatic epithelium; DF, dominant tumor focus; AF, additional tumor focus; LN, metastatic lymph node; R, both alleles retained; L, allelic loss; NA, not applicable; NI, not informative; ND, not determined.
[b] Cases for which number of foci in prostate gland could not be determined.

Table 3 *Allelic loss frequencies in histological components of the prostate*

Allelic loss frequency is defined as number of specimens exhibiting loss/number of specimens informative at the locus examined.

	8p loss	10q loss	16q loss	12p loss
Normal	0/14 (0%)	0/17 (0%)	0/12 (0%)	0/12 (0%)
PIN	2/7 (29%)	2/8 (25%)	1/5 (20%)	0/5 (0%)
Primary tumors[a]	4/14 (29%)	3/16 (18%)	5/12 (42%)	0/12 (0%)
Individual foci[b]				
Combined Gleason score 4, 5, 6	1/7 (14%)	1/15 (7%)	1/8 (13%)	0/9 (0%)
Combined Gleason score 7, 8, 9	3/15 (20%)	2/21 (10%)	3/13 (23%)	0/14 (0%)
Metastases	3/12 (25%)	2/17 (12%)	1/12 (8%)	0/12 (0%)

[a] Collective allelic loss in the prostate gland of each case examined.

[b] Allelic loss in individual tumor foci per prostate gland (this number exceeds the number of cases examined when more than one tumor focus was examined per gland).

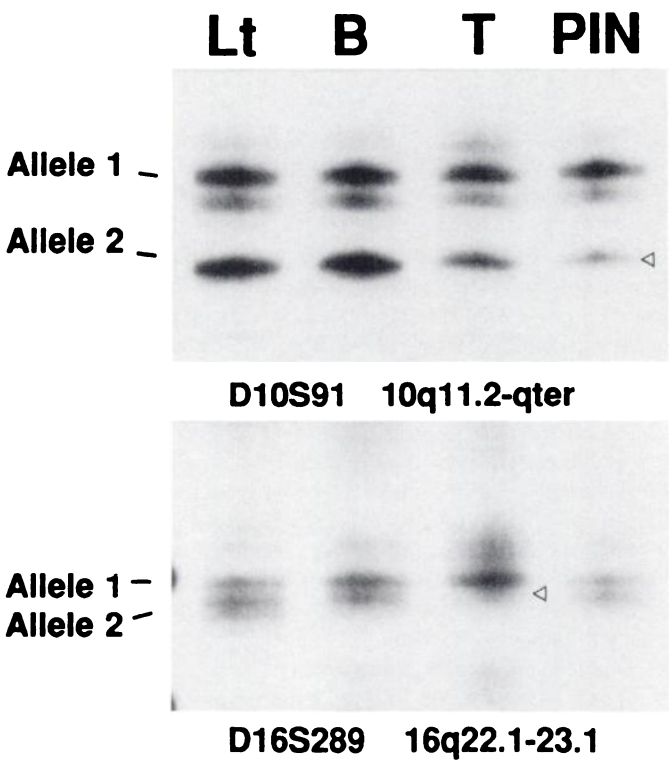


Fig. 2. Allelic loss of 10q and 16q sequences demonstrated by PCR analysis. DNA purified from microdissected lymph node metastasis (Lt), benign epithelium (B), tumor (T), and PIN tissue from patient 14 was amplified for sequences containing microsatellite repeats at the *D10S91* locus (*top*) or *D16S289* locus (*bottom*). The PCR reaction products were autoradiographed for 16 h. Allelic loss is evident for the PIN specimen at the 10q locus (*top*, allele 2, *arrowhead*) and for the tumor specimen at the 16q locus (*bottom*, allele 2, *arrowhead*). Although some loss is also evident for the tumor specimen at the 10q locus, it did not meet the criteria of at least 50% loss and was therefore not considered significant loss.

histological components within more complex prostatic tumors demonstrate greater genetic heterogeneity at the 8p but not 10q or 16q loci than those within simpler prostates (Table 4).

DISCUSSION

The molecular events responsible for the evolution of prostatic cancer are poorly understood. The relatively recent and concentrated efforts to define genetic aberrations in this tumor system have been compromised by the rather complex and heterogenous growth pattern of prostatic adenocarcinoma. Our study is one of the first attempts to characterize allelic loss for several chromosomal loci in anatomically separate, histologically different, primary tissue components in patients with locally metastasized prostatic carcinoma. We explored whether a recognizable pattern of genetic aberration could be established between the putative premalignant lesions of high-grade PIN, well to moderately and poorly differentiated adenocarcinoma, and metastatic deposits in regional lymph nodes. As

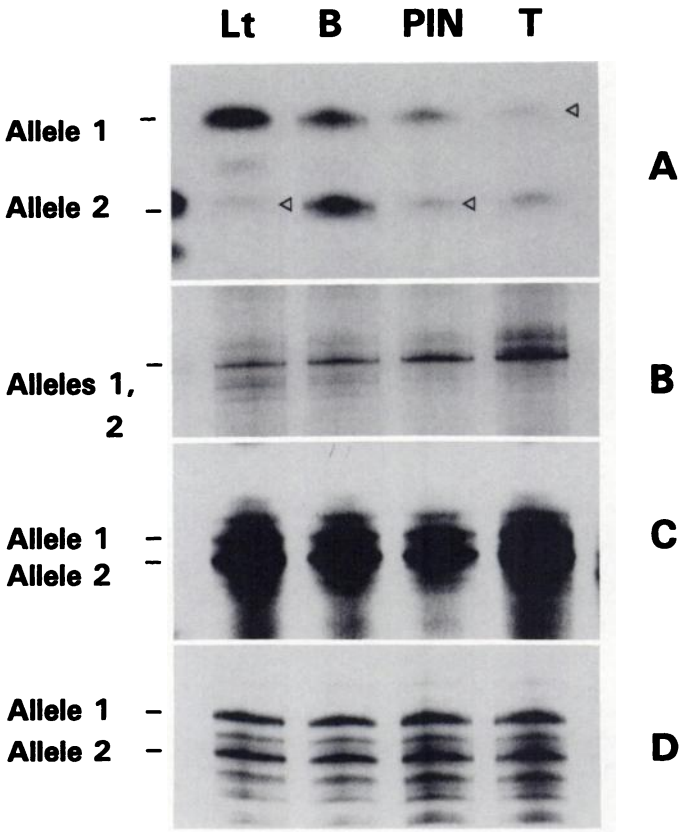


Fig. 3. Allelic loss of 8p sequences demonstrated by PCR analysis. DNA purified from microdissected lymph node metastasis (Lt), benign epithelium (B), PIN, and tumor (T) tissue from patient 18 was amplified for sequences containing microsatellite repeats at the *LPL* locus (*A*), *D16S289* locus (*B*), *D10S91* locus (*C*), or *F8VWF* locus (*D*). The PCR reaction products were autoradiographed for 16 h. Allelic loss is evident for the Lt and PIN specimens (*A*, allele 2, *arrowhead*) and for the tumor (*A*, allele 1, *arrowhead*) at the *LPL* locus. Allelic loss is not evident at the *D10S91* or *F8VWF* loci (*C* and *D*, respectively). The specimens were not informative at the *D16S89* locus (*B*).

Table 4 *Comparison of allelic loss patterns in multisampled prostates*

Data is from cases 1–16.

	<3 tumor foci/gland			≥3 tumor foci/gland		
	8p	10q	16q	8p	10q	16q
Dominant tumor focus and metastasis						
Conordant[a]	5	4	2	3	7	6
Discordant	0	1	1	3	2	1
Additional tumor foci and metastasis						
Concordant	0	3	1	3	5	6
Discordant	0	0	1	2	2	0
Dominant and additional tumor foci						
Concordant	0	2	1	4	6	5
Discordant	0	0	0	2	2	1
PIN and metastasis						
Concordant	2	0	1	2	4	3
Discordant	0	1	0	2	1	1
PIN and dominant tumor focus						
Concordant	2	0	0	2	4	2
Discordant	0	1	1	2	1	2

[a] Concordant, similar allelic loss patterns in compared informative specimens (*e.g.*, the compared specimens both retained or both lost alleles at the indicated locus); discordant, dissimilar allelic loss patterns in compared informative specimens (*e.g.*, alleles are lost in one but retained in the other compared specimen at the indicated locus).

expected, given the pathological stage analyzed, most tumors were extensive with a large volume and high Gleason's score. In 10 of the 19 cases, however, sampling two anatomically separate primary tumor nodules, benign or benign hyperplastic prostatic tissue, and metastatic deposits was possible.

Although a trend toward increased frequency of allelic loss for 8p, 10q, and 16q was observed with increasing tumor grade, no significant correlations were observed (Table 3). Therefore, it is unclear whether allelic loss of these loci potentially represent "early" or "late" events in prostatic tumorigenesis. Furthermore, the observation of similar or even reduced allelic loss frequencies at 8p, 10q, and 16q in metastases compared to primary tumors may indicate that these metastases arose from tumor foci other than the dominant tumor nodule in the gland. Conversely, allelic loss events apparent in the dominant tumor nodule but not the metastasis from the same patient may suggest that the allelic loss events occurred after metastasis or that the metastasis arose from another, unsampled, tumor focus. In any of these scenarios, it is apparent that accumulation of genetic aberrations may differ in primary and metastatic lesions in the prostate. Perhaps, complete sampling of all tumor foci in the prostate could better address this point.

We observed differences in sequence dosage at 8p between different histological components from the same cases when comparing metastases and dominant tumor nodule, metastases and additional tumor foci, dominant and additional tumor foci, PIN and metastases, or PIN and dominant tumor nodule (Table 4). These differences, or discordancies, were most pronounced for 8p loss in cases in which the prostate contained ≥ 3 tumor. These result suggest that histological components within more complex prostatic tumors may demonstrate greater genetic heterogeneity at the 8p locus, but not 10q or 16q loci, compared to those within simpler prostates.

Examination of allelic loss patterns in PIN lesions revealed frequencies similar to those observed in primary tumors (Table 3). This indicates that high-grade PIN shares certain genetic aberrations noted in prostatic carcinoma.

In summary, our findings demonstrate that the morphological heterogeneity evident in prostatic neoplasia and premalignant lesions may be reflected at the genetic level, *e.g.*, as heterogeneity in loss of 8p sequences. These studies show that allelic losses at 8p, 10q, or 16q do not solely characterize more advanced or metastasizing tumors but are also found with considerable frequency in putative premalignant lesions. Furthermore, genetic heterogeneity of 8p allelic loss shows increasing complexity in a fashion similar to that established morphologically, *e.g.*, it is most frequent in complex prostatic tumors containing ≥ 3 tumor foci rather than simple tumors containing only one or two distinct tumor foci. Finally, our results suggest that lymph node metastases may be genetically related to either the dominant or additional primary tumor foci in more complex prostates and that the accumulation of genetic aberrations may differ in primary and metastatic lesions.

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