

# Allelic Imbalance in the Clonal Evolution of Prostate Carcinoma

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**BACKGROUND.** To understand better the genetic basis of the clonal evolution of prostate carcinoma, the authors analyzed the pattern of allelic loss in 25 matched primary and metastatic prostate tumors.

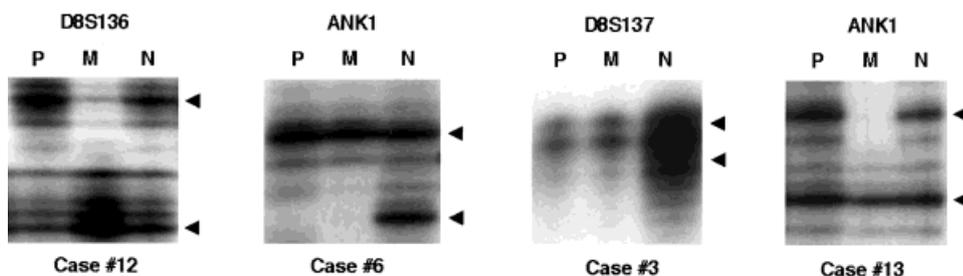
**METHODS.** Twenty-five cases were selected from the surgical pathology files of the Mayo Clinic from patients who had undergone radical retropubic prostatectomy and bilateral lymphadenectomy between 1987-1991. All patients had regional lymph node metastases at the time of surgery. DNA samples for the analysis of allelic loss pattern were prepared from primary tumors and matched synchronous lymph node metastases by tissue microdissection. The oligonucleotide primer pairs for the microsatellite DNA markers were D8S133, D8S136, D8S137, ANK1 on chromosome 8p12-21, LPLTET on chromosome 8p22, and D17S855 (intragenic to the BRCA1 gene) on chromosome 17q21. One case was not informative at any of the loci tested and was excluded from further analysis.

**RESULTS.** The overall frequency of allelic imbalance was 79% in primary tumors and 88% in paired metastases. Of 24 informative cases, 14 patients (58%) showed the same pattern of allelic loss or retention in matched primary and metastatic tumors at all marker locus; discordant allelic loss was observed in the remaining 10 patients (42%). Four patients showed loss of the same allele at one or more marker loci in both primary and metastatic tumors, but discordant allelic loss was observed at other marker loci. Five patients showed allelic loss in at least one genetic marker in the metastatic tumor but not in its matched primary tumor. Five patients displayed loss of one allele at one or more marker loci in a primary tumor but not in the matched metastases. There was no significant difference in the frequency of allelic imbalance between primary and metastatic tumors at any marker analyzed ( $P > 0.05$ ).

**CONCLUSIONS.** These data suggest that different patterns of allelic deletion may be acquired during cancer progression to metastases. The differences in genetic composition between primary prostate carcinoma and its metastases may be related to intrinsic cancer heterogeneity, overall genetic instability, and clonal divergence. *Cancer* 1999;85:2017-22. © 1999 American Cancer Society.

**KEYWORDS:** prostate, metastases, allelic imbalance, microdissection, progression.

The most lethal aspect of cancer is the ability of tumor cells to spread to distant organs, because the majority of cancer-related deaths are attributed to the progressive growth of metastases.<sup>1,2</sup> Our understanding of molecular mechanisms underlying metastatic progression is limited. Recent advances in tissue microdissection techniques permit the selective procurement of tumor cell populations from paraffin embedded archival material for genetic analysis.<sup>3,4</sup> Detailed characterization and comparison of genetic alterations of biologically distinct tumor cell subpopulations may provide information regarding cancer progression and clonal evolution of cancer.<sup>5-7</sup>



**FIGURE 1.** Representative results of allelic imbalance in primary tumor and its matched metastases. DNA was prepared from primary tumor (P), lymph node metastases (M), and normal tissue (N) (control) from the same patient by tissue microdissection and amplified using polymerase chain reaction. Case 12 showed loss of different alleles at DNA marker D8S136. The same allele (lower allele) was lost in both the primary tumor and its matched metastases for Cases 6 and 3. Case 13 showed allelic deletion in metastases that was not present in the primary tumor. Arrows point to the allelic band in each case.

Recent data<sup>5-7</sup> demonstrated that similar genetic alterations often were observed in both prostate carcinoma and prostatic intraepithelial neoplasia (PIN). Prostate carcinoma usually has more chromosomal anomalies than matched PIN foci.<sup>5,6</sup> By regional mapping of the whole mount prostate, Qian et al.<sup>5</sup> and Jenkins et al.<sup>6</sup> demonstrated that one or more foci of tumor shared chromosomal anomalies with paired lymph node metastases, suggesting that a single focus of carcinoma may give rise to metastases.<sup>5,6</sup> Previous studies reported that the pattern of allelic loss in primary breast carcinoma was maintained during disease progression to metastases and identical allelic loss in primary tumor was conserved in paired metastatic carcinoma, suggesting that the majority of allelic imbalance (AI) occurs prior to metastases.<sup>8,9</sup> It is unknown whether allelic loss can be acquired after disease progression to metastases and whether the pattern of AI in primary prostate carcinoma is preserved in the matched metastatic tumor. In the current study, we analyzed the pattern of AI on chromosome 8p12-22, the region of putative tumor suppressor gene, and on chromosome 17q21,<sup>10-12</sup> the BRCA locus,<sup>13</sup> in primary prostate carcinoma specimens and their matched metastases using tissue microdissection.

## METHODS

Twenty-five cases were selected from the surgical pathology files of the Mayo Clinic from patients who had undergone radical retropubic prostatectomy and bilateral lymphadenectomy between 1987-1991. These cases were chosen randomly based on the availability of tissues from matched primary tumor and lymph node metastases. All patients had regional lymph node metastases at the time of surgery. Patients

ranged in age from 53-74 years (mean, 67 years; median, 66 years). Grading of the primary tumor from radical prostatectomy specimens was performed according to the Gleason system.<sup>14</sup> Pure populations of neoplastic cells and normal tissue were microdissected directly from formalin fixed, paraffin embedded tissue under direct light microscopic visualization, as previously described.<sup>3,10,15,16</sup> Multiple areas of the largest tumor foci were sampled for analysis. DNA samples for the analysis of allelic loss pattern were prepared from primary tumor and matched synchronous lymph node metastases. The oligonucleotide primer pairs for the microsatellite DNA markers were: D8S133, D8S136, D8S137, ANK1 on chromosome 8p12-21, LPLTET on chromosome 8p22, and D17S855 on chromosome 17q21 (Research Genetics, Huntsville, AL).<sup>11</sup> The D17S855 locus is intragenic to the BRCA1 gene.<sup>13</sup> Polymerase chain reaction (PCR) amplification and gel electrophoresis were performed as described previously.<sup>3,10,15,16</sup> The criterion for AI was complete or nearly complete absence of one allele in tumor DNA as defined by direct visualization.<sup>15,16</sup> PCR reactions for each polymorphic microsatellite marker were repeated at least twice, and the same results were obtained. Statistical analysis was performed using the Fisher exact test (two-sided), with *P* values < 0.05 considered statistically significant.

## RESULTS

We analyzed the pattern of allelic loss in 25 matched primary and metastatic tumors with 6 polymorphic microsatellite markers on chromosome 8p12-21, 8p22, and 17q21 (Fig. 1) (Table 1). DNA samples were extracted from archival paraffin embedded tissue by microdissection and used for PCR amplification.

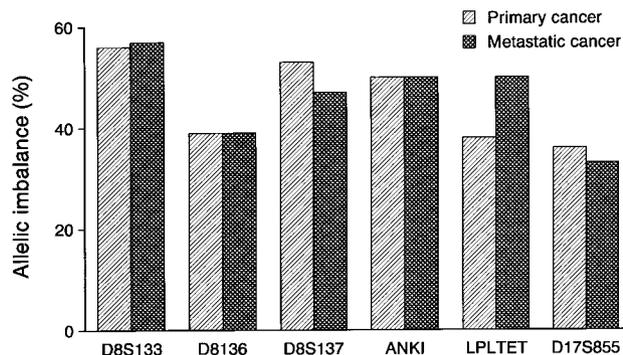
The overall frequency of AI was 79% (19 of 24

**TABLE 1**  
**Comparison of Allelic Loss Pattern in Matched Primary and Metastatic Prostate Carcinoma Specimens from the Same Patient**

Case no.	Tumor	Age (yrs)	Grade	Allelic loss <sup>a</sup>					
				D8S133	D8S136	D8S137	ANK1	LPLTET	D17S855
1	P	70	3 + 4	NI	2	2	NI	NI	NL
	M			NI	2	2	NI	NI	NL
2	P	66	3 + 3	NI	NL	NL	NL	1	NL
	M			NI	NL	NL	NL	1	NL
3	P	74	3 + 4	NL	NL	2	2	NI	1
	M			NL	NL	2	2	NI	1
4	P	69	4 + 3	2	NI	NI	NI	NL	NE
	M			1	NI	NI	NI	NL	NE
5	P	66	4 + 3	NL	1	NL	NI	1	NL
	M			NL	1	NL	NI	1	NL
6	P	70	5 + 4	1	2	NE	2	NL	NL
	M			1	2	NE	2	NL	1
7	P	69	4 + 3	NI	NL	NL	NI	NL	1
	M			NI	NL	NE	NI	NL	1
8	P	64	4 + 3	NE	NL	NI	NI	NI	NE
	M			NE	NL	NI	NI	NI	NE
9	P	69	3 + 3	NE	NI	NE	NI	NI	NE
	M			NE	NI	NE	NI	NI	NE
10	P	63	5 + 4	NL	NL	NL	NI	NL	NI
	M			NL	NL	NL	NI	NL	NI
11	P	70	3 + 4	2	2	2	NL	1	NI
	M			2	2	2	NL	1	NI
12	P	65	3 + 4	2	2	2	NI	NL	NL
	M			1	1	1	NI	1	NL
13	P	74	3 + 4	NI	2	NI	NL	NL	NL
	M			NI	NL	NI	1	NL	NL
14	P	64	5 + 4	1	NI	NL	2	1	1
	M			1	NI	NL	2	1	1
15	P	62	4 + 3	NE	1	NI	NL	NL	NI
	M			NE	1	NI	1	2	NI
16	P	57	4 + 5	NI	NL	NI	NL	NI	NI
	M			NI	2	NI	NL	NI	NI
17	P	71	5 + 5	NL	NL	NL	NL	NI	NI
	M			NL	NL	NE	NL	NI	NI
18	P	61	5 + 4	1	NI	1	1	NI	NI
	M			1	NI	1	1	NI	NI
19	P	65	5 + 4	NI	NI	1	1	NI	NI
	M			NI	NI	1	NL	NI	NI
20	P	53	4 + 3	NI	NI	NI	1	1	NL
	M			NI	NI	NI	1	1	NL
21	P	63	4 + 3	2	NI	2	1	1	2
	M			2	NI	2	1	1	NL
22	P	62	5 + 4	2	NL	NE	NI	NL	NI
	M			2	NL	NE	NI	NL	NI
23	P	67	3 + 4	NL	NL	NL	NL	NL	NL
	M			NL	NL	NL	NL	NL	NL
24	P	58	4 + 3	2	NL	1	2	NI	NL
	M			NL	NL	NL	NL	NI	1
25	P	68	3 + 5	NI	NL	NL	NL	NL	2
	M			NI	NL	NL	NL	NL	NL

P: primary tumor; M: synchronous lymph node metastases; NI: noninformative; NL: no loss of alleles; NE: not evaluable.

<sup>a</sup> In the columns headed "allelic loss" "1" indicates loss of an upper allele and "2" indicates loss of a lower allele.



**FIGURE 2.** Comparison of allelic imbalance at six polymorphic microsatellite markers between matched primary tumor and metastases. There was no significant difference in the distribution of allelic imbalance at any marker analyzed ( $P > 0.05$ ).

informative cases) in primary tumors and 88% (21 of 24 informative cases) in paired metastatic tumors. The frequency of allelic loss in the primary tumors was 56% with D8S133, 39% with D8S136, 53% with D8S137, 50% with ANK1, 38% with LPLTET, and 36% with D17S855. The frequency of allelic loss in matched metastatic tumors was 57% with D8S133, 39% with D8S136, 47% with D8S137, 50% with ANK1, 50% with LPLTET, and 33% with D17S855. There was no significant difference in the frequency of allelic loss between primary and metastatic tumors at any marker locus analyzed ( $P > 0.05$ ) (Fig. 2). None showed evidence of homozygous loss for any DNA marker analyzed. Four patients showed no allelic loss in the metastatic tumor at any given marker locus examined (Cases 8, 10, 17, and 23). One case was not informative at any of the loci tested and was excluded for further analysis (Case 9).

An identical pattern of AI (allelic loss or retention) was observed in matched primary and metastatic tumors in 14 cases (58%) (Cases 1, 2, 3, 5, 7, 8, 10, 11, 14, 17, 18, 20, 22, and 23), suggesting clonal origin of the tumor cells. Discordant allelic loss at  $\geq 1$  marker locus was observed in the remaining 10 cases (42%). Four cases (Cases 6, 15, 19, and 21) showed loss of the same allele at  $\geq 1$  marker loci in both the primary and metastatic tumor, but discordant allelic loss was observed at other marker loci. Five cases (Cases 6, 13, 15, 16, and 24) showed allelic loss in at least one genetic marker in the metastatic tumor, but not in its matched primary tumor. For example, Case 6 showed allelic loss in the metastatic tumor at D17S855 that was not recognized in the primary tumor. In addition, identical allelic loss was observed at three other marker loci (D8S133, D8S136, and ANK1). Although these results could be explained either by different clonality of the tumor cells or by changes consistent with genetic evo-

lution and clonal divergence during cancer progression, seven cases presented more diverse results; five cases (Cases 13, 19, 21, 24, and 25) showed loss of one allele at one or more marker loci in the primary tumor but not in the matched metastases. For example, Case 21 showed loss of the same allele in the primary tumor and matched metastases at four marker loci (D8S133, D8S137, D8S137, ANK1, and LPLTET), but allelic loss was observed only in the primary tumor at locus D17S855. Furthermore, two cases (Cases 4 and 12) demonstrated loss of different alleles at the same marker locus in matched primary and metastatic tumors. In Case 4, the primary tumor showed loss of the lower allele with marker D8S133, but loss of the upper allele was observed in the matched metastatic tumor with the same DNA marker. Case 12 displayed loss of different alleles in the primary versus metastatic tumor with three markers (D8S133, D8S136, and D8S137).

## DISCUSSION

We compared the pattern of AI in 25 matched primary and metastatic prostate carcinoma tumors using tissue microdissection and PCR amplification of microsatellite markers on chromosome 8p12-22, the region of a putative tumor suppressor gene,<sup>10-12</sup> and on chromosome 17q21,<sup>13</sup> the BRCA1 locus. We found identical patterns of allelic loss/retention in the primary tumor and matched metastases in 14 of 24 informative cases (58%). However, a heterogeneous pattern of allelic loss was observed in other patients. Allelic loss at some marker loci that is present in primary tumors may not be observed in matched lymph node metastases, whereas allelic loss recognized in a metastatic tumor may not be observed in its primary tumor. The different genetic composition of primary prostate carcinoma and its metastases may reflect tumor heterogeneity and clonal divergence. We were unable to detect any specific marker that was associated with metastatic phenotype. These findings suggest that allelic loss at the marker loci we analyzed may be attributed to randomly acquired genetic alterations due to tumor genomic instability.

To our knowledge, few studies have compared allelic loss in synchronous primary tumors and lymph node metastases. In the study by Sakr et al.<sup>17</sup> of 19 matched primary and metastatic prostate carcinoma tumors only 4 cases showed allelic loss in the metastatic tumor at any given marker locus examined, which limited further analysis. In contrast, allelic loss at  $\geq 1$  DNA marker locus was observed in 88% of metastatic tumors in the current study. This may be attributed to differences in the microdissection technique, DNA markers selected, and the patient popu-

lation. Our findings also differed from other genetic studies of matched primary and metastatic breast carcinoma. Bonsing et al. found that every allelic imbalance present in a primary tumor was present in all DNA samples of related lymph node metastases, suggesting that an advanced primary breast carcinoma is comprised of a clonal tumor cell population with an established complement of AI in all parts of the primary tumor and in the related lymph node metastases at the time of diagnosis.<sup>8</sup> Similar results were reported in a study by Chen et al. in which AI observed in primary breast carcinoma was always observed in the matched lymph node metastases of the same patient.<sup>9</sup> In our study, discordant patterns of allelic loss were observed in a substantial proportion of cases (42%), including cases with allelic loss versus retained heterozygosity in primary and metastatic tumors, as well as cases with loss of different alleles. Therefore it is possible that the metastatic tumor cells may be derived from separate tumor foci that were not sampled. Previous studies showed that separate tumors often have different genetic compositions, and multiple tumors from the same patient may arise independently.<sup>4-7, 16-18</sup> As documented by Qian et al., separate small tumors in the prostate may give rise to distant metastases.<sup>5</sup> Whole mount sections from entirely embedded prostate glands would allow for the accurate assessment of the multifocality of prostate carcinoma.<sup>5-7,16,18</sup>

Our data also suggested the possibility that additional allelic loss may be acquired after progression to metastases. It generally is accepted that identical allelic losses at multiple loci provide evidence of a clonal relation.<sup>7,15,16,19,20</sup> In this study, four patients showed loss of the same allele at one or more marker loci in both primary and metastatic tumors, but a different allelic loss pattern was observed at other marker loci. Case 21 showed loss of the same allele in the primary tumor and its matched metastases at four marker loci (D8S133, D8S137, ANK1, and LPLTET), but allelic loss was observed only in a primary tumor at locus D17S855, suggesting that acquisition of AI at this locus in the primary tumor may occur after it metastasizes. Similarly, allelic loss in the metastases at locus D18S155 in Case 6 may have been acquired after the primary tumor had metastasized because the other three marker loci showed identical allelic loss in the primary and metastatic tumors. It is unlikely that failure to detect allelic loss in the primary or metastatic tumors in this case or other cases was due to contamination of normal cells because allelic deletion frequently was detected at another marker locus using the same DNA sample from the same patient. These findings suggest that allelic loss may be acquired in

both primary and metastatic tumors after cancer progression.

Genetic alterations that are crucial for carcinogenesis occur early in tumor development. Accordingly, these genetic changes will be maintained in the subsequent clonal evolution. Previous studies have found frequent allelic loss on chromosome 8p in both PIN and concurrent prostate carcinoma,<sup>5,7,10,17</sup> suggesting that allelic loss in this region is an early molecular event during prostate carcinogenesis. Therefore, it would be expected that a highly conserved pattern of allelic loss on chromosomal 8p would be observed in different stages of cancer progression. However, our data indicated a consistently discordant pattern of allelic loss/retention in a substantial proportion of matched primary and metastatic tumors. Furthermore, a concordant pattern of allelic loss/retention by itself does not exclude different clonality. A recent study demonstrated genetic heterogeneity of prostate carcinoma metastases, suggesting a complex genetic relation between various clonal lineages of prostate carcinoma during tumor progression.<sup>21</sup>

Our data suggest that different complements of AI may be acquired after cancer progression and subsequent metastases. We believe these findings have implications for understanding the molecular basis of metastatic progression in prostate carcinoma. Both primary and metastatic tumors may undergo further genetic evolution and the primary tumor may be as genetically advanced as its metastases. The differences in genetic constitution between primary prostate carcinoma and its metastases may reflect tumor heterogeneity, overall genetic instability, and clonal divergence.

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