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Loss of Heterozygosity at 7q31 Is a Frequent and Early Event in Prostate Cancer[1]

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ABSTRACT

It is widely accepted that an accumulation of genetic alterations plays an important role in the genesis of human cancers, but little is known about prostate cancer in this respect. Recent studies have identified regions on chromosome arms 8p, 10q, 16q, and 18q that are frequently deleted in human prostate cancer. We have previously described a loss of heterozygosity (LOH) at the *Met* locus on chromosome band 7q31 in a study of 20 localized prostate tumors. To determine whether a region on the 7q arm is important in the initiation and/or progression of prostate cancer, prostate tissue from 13 patients with confined prostate tumors, 17 with local extracapsular extension, and 13 with metastatic forms were analyzed for LOH, using a DNA probe for RFLP (pMetH) and 8 CA microsatellite repeats (7 on 7q21–q33 and 1 on 7p). Twenty (47%) of the 43 cases studied showed LOH at one or more 7q loci.

The most frequently deleted region was chromosome 7q31.1–7q31.2, whereas the centromeric locus on 7q21 was generally conserved. The percentage of LOH was normally distributed around the *D7S480* locus. Moreover, the rate of LOH in the 7q31 region was lower in metastatic tumors than in localized tumors. These results strongly suggest the presence of a tumor suppressor gene on the chromosome band 7q31 with an important role in the early stages of prostate cancer.

INTRODUCTION

Prostate cancer is the most frequently diagnosed solid tumor and the second cause of death from cancer among men in western countries. Although little is known of the precise molecular bases, stepwise accumulation of genetic alterations is thought to be involved in the genesis and progression of prostate tumors, by analogy with a well-established model of colorectal carcinogenesis (1). The most consistent genetic alterations in adenocarcinoma of the prostate are LOH[4] involving chromosome arms 8p, 10q, 16q, and 18q, pointing to the presence of tumor suppressor genes in these regions and their involvement in prostate carcinogenesis (2–8). In a previous study, we described LOH of the c-*met* gene (33%) located on chromosome band 7q31 (8), whereas Zenklusen *et al.* (9), using microsatellite markers, recently reported a high frequency of allelic loss in the same region in clinically localized prostate tumors. To refine our knowledge of such a genetic region on the 7q arm and to establish clinical-pathological correlations, we futher supplemented the RFLP marker c-*met* with microsatellite repeat polymorphisms (10) on chromosome 7 to analyze allelic imbalance in a larger number of clinically localized tumors and metastatic forms.

MATERIALS AND METHODS

Patients and Samples. Forty-three prostate tumor specimens were obtained from patients undergoing surgery at St. Louis and Bichat hospitals in Paris, and Morvan Hospital in Brest. The samples were obtained from locally confined and local extracapsular tumors by means of radical prostatectomy or needle biopsy, whereas those from patients with regional lymph node involvement or distant metastases were obtained by transurethral resection. The samples were examined histologically to confirm the presence of tumor cells. A sample was considered suitable for DNA analysis if the proportion of tumor cells was 60% or more. All suitable samples were frozen in liquid nitrogen until high molecular weight DNA extraction. The histological diagnosis, Gleason score (11), and pathological tumor stage according to the TNM classification of prostate cancer (12) were determined in each case during a routine clinical workup after surgery.

The Gleason score for the 43 primary tumors ranged from 5 to 9. By combining the Gleason score with the pathological stage, we confirmed the well-established positive link between grade and stage.

The tumors were subdivided into three groups corresponding to the TNM staging system as described by Boswick *et al.* (13).

Group A patients had disease limited to the prostate (n = 13; 30%); group B patients had local extracapsular extension (n = 17; 40%); and group C patients had regional lymph node involvement or distant metastases (n = 13; 30%). Microscopic lymph node metastases were seen in 6 of the 43 cases, and distant metastases were observed in 7 cases.

We used one DNA probe for RFLP polymorphism (pMetH) and eight microsatellite markers on chromosome 7

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[4] The abbreviations used are: LOH, loss of heterozygosity; SCDR, smallest common deleted region.

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Table 1 Allelic loss on chromosome 7 in a series of 43 human prostate tumors

Markers	Polymorphism type	Probe	Enzyme	Location	Ref.	Allelic losses/ informative cases (all tumors)
D7S435	CA repeat	mfd20		7p	(14)	6/34 (18%)
D7S440	CA repeat	mfd50		7q11–q21	(14)	4/35 (11%)
Met	RFLP	pMetH	*Taq* I	7q31.1	ATCC[a]	5/21 (24%)
D7S486	CA repeat	AFM098xg9		7q31.1	(15)	11/34 (32%)
D7S522	CA repeat	AFM242yc3		7q31.1	(15)	9/25 (36%)
D7S480	CA repeat	AFM042xh10		7q31.2	(15)	15/33 (46%)
D7S650	CA repeat	AFM240zh10		7q31.2	(15)	10/25 (40%)
D7S490	CA repeat	AFM150yg7		7q31.2	(15)	13/33 (39%)
D7S495	CA repeat	AFM168xc3		7q33	(15)	12/31 (39%)

[a] ATTC, American Type Culture Collection.

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(seven on 7q and one on 7p) to screen the 43 samples. Table 1 gives details of the loci investigated and their corresponding chromosomal locations (14–16).

Peripheral blood leukocytes were used as a source of normal DNA in each case.

Southern Blot Analysis. Frozen tissue samples were ground in liquid nitrogen to a fine powder using a mortar and pestle. High molecular weight DNA was prepared by proteinase K digestion and phenol-chloroform extraction from both blood and tissue samples. Ten μg of genomic DNA from each sample were digested with *Taq*I and fractionated by electrophoresis on a 0.8% agarose gel.

Leukocyte and tumor DNA from each patient were analyzed in adjacent tracks. DNA was then transferred to nylon membrane filters according to standard blotting procedures. The DNA probe was labeled with [^{32}P]dCTP by using a random primer labeling system. The membrane filters were hybridized overnight at 65°C with the denatured labeled probe, washed, and autoradiographed at −80°C for an appropriate period.

Detection of Microsatellite Markers by PCR. PCR was run in a total volume of 50 μl, with 50 ng genomic DNA, 20 mM each primer, 1.5 mM $MgCl_2$, 0.1 mM each deoxynucleotide triphosphate, and 1 unit *Taq* DNA polymerase. Microsatellite markers were assayed by PCR amplification of genomic DNA. Standard DNA amplification conditions were as follows: 27 cycles of denaturation for 1 min at 94°C, 1 min of annealing at 55°C (*D7S435* and *D7S440*), 60°C (*D7S522*, *D7S486*, *D7S480*, *D7S650*, and *D7S490*), or 62°C (*D7S495*), and 30-s extension at 72°C. A final extension step at 72°C was lengthened to 5 min. Products were diluted 1:3 in denaturing loading buffer and heat denatured; then 1.5-μl aliquots of each sample were loaded on 6% acrylamide gels containing 7.5 M urea. DNA was then transferred to nylon membrane filters. The CA repeat probe was labeled with [^{32}P]dCTP by using terminal deoxynucleotidyl transferase. The membrane filters were hybridized overnight at 42°C with the labeled probe, washed, and autoradiographed at −80°C for an appropriate period.

Determination of Allele Loss. Allelic loss can only be identified in "informative" cases. Normal DNA samples polymorphic at a given locus were considered informative, whereas homozygotes were considered uninformative. The signal intensity of fragments was determined by means of densitometry and/or visual examination (three observers). LOH was considered to occur when the intensity of one of the two alleles in the tumor DNA was <30% of that in the corresponding normal tissue DNA for a given amount of DNA.

RESULTS

We analyzed normal DNA (peripheral blood lymphocytes) and autologous tumor DNA from 43 patients with prostate cancer, using seven polymorphic probes for the long arm of chromosome 7 and one polymorphic marker mapping to the short arm to obtain an overall picture of chromosome 7 (Table 1).

All patients were informative for two or more loci on 7q. Allelic imbalance on at least one locus was found in 20 (47%) of the 43 tumor DNAs. The informative patients in whom an imbalance between the two alleles in the tumor DNA track was observed (with the RFLP marker c-*met*) showed that the nature of the imbalance was classical LOH. Only one tumor (tumor 13) had lost a single locus (*D7S480*), whereas the other 19 had lost multiple adjacent markers on the long arm of chromosome 7. The percentage of tumors with allelic loss at each locus is shown in Table 1, with a maximum rate of 46% at *D7S480* in 7q31.1–31.2.

Of these 20 7q-altered tumors, 13 (65%) showed partial (interstitial and/or telomeric) alterations on 7q, whereas 7 (35%) showed LOH at all informative loci tested on 7q. To obtain an overall picture of chromosome 7, all tumors were tested for 7p retention by using a microsatellite marker, D7S435. Three of the seven tumors with an entire deletion of 7q also showed a deletion of the *D7S435* locus, indicating monosomy 7, and three showed allelic retention on 7p, suggesting the loss of the entire long arm of chromosome 7q; the last sample (tumor 36) was not informative and could have monosomy 7 or monosomy 7q.

LOH on 7p was also found in two tumors that showed partial deletion of 7q (tumors 26 and 37) and in one tumor with no 7q alterations (tumor 47).

Interestingly, 12 of these 13 tumors with partial deletions on 7q showed LOH at loci *D7S480* and/or *D7S650*, suggesting the presence of a SCDR. Fig. 1 summarizes LOH data of these tumors; Fig. 2 shows examples of the most common patterns of genetic changes. Six of these 12 tumors were in group A, 4 in group B, and 2 in group C. The last partially 7q-deleted tumor (tumor 37, also in group A) showed both allelic loss on 7p, and

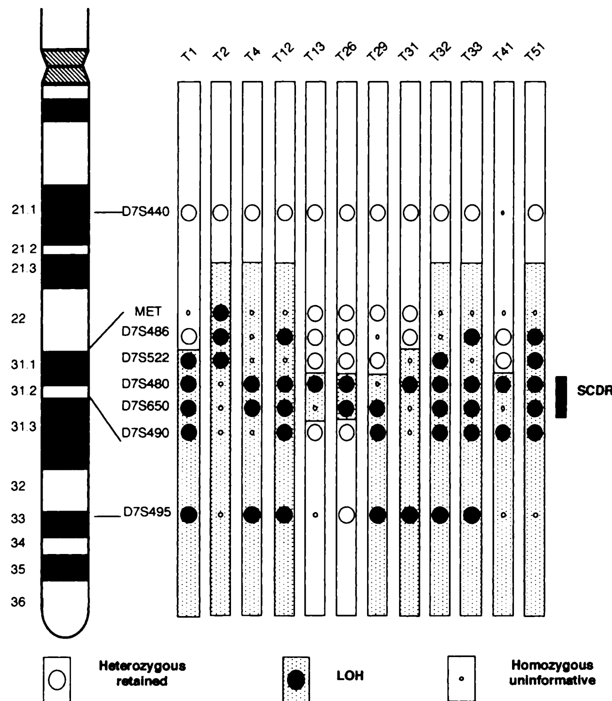

Fig. 1 LOH analysis at various chromosome arm 7q loci in prostate cancer. Only tumors showing partial 7q LOH are illustrated. *Left*, probes used and their relative locations. *Circles*, informative loci; *dots*, homozygous uninformative loci. *Shaded area*, maximum extent of the LOH, as defined by one or more probes. *Right*, SCDR.

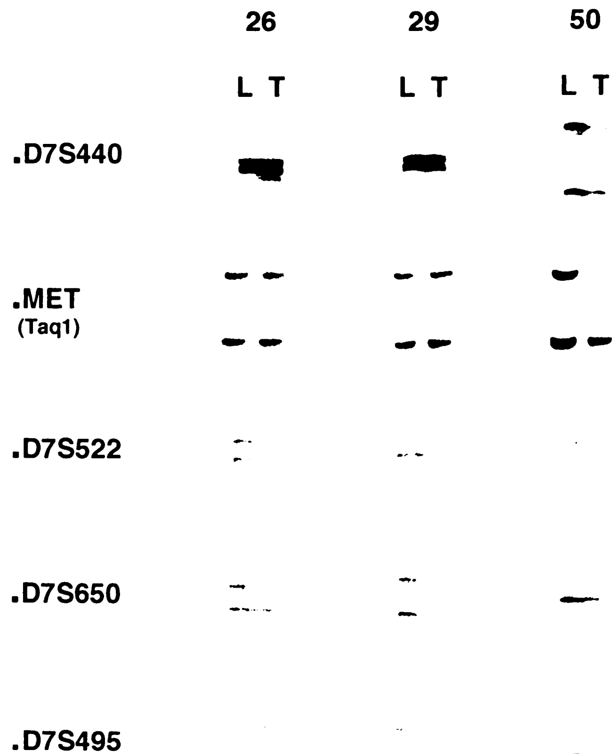

Fig. 2 Representative results obtained with polymorphic markers. *L* and *T*, matched DNA samples isolated from peripheral leukocytes and tumor tissue, respectively. LOH, in informative cases, is defined as the loss of one of the two alleles in the tumor compared with normal somatic DNA. The faint signals in tumor DNA might be due to either contaminating normal tissue or tumor heterogeneity. Tumors 26, 29, and 50 are shown with respect to D7S440, D7S522, D7S650, and D7S495 (microsatellite markers) and c-*met* (a RFLP marker). Case 26 shows an interstitial deletion (LOH for D7S650 and retention for D7S440, c-*met*, D7S522, and D7S495). Case 29 shows a partial deletion (LOH for D7S650 and D7S495 and retention for D7S440, c-*met*, and D7S522). Case 50 shows a deletion of the entire chromosome arm 7q (LOH for D7S440, c-*met*, D7S522, and D7S495; D7S650 is uninformative).

proximal loss on 7q, which did not overlap with the *D7S480* locus. Allelic loss at one or more loci in the 7q31 region was analyzed according to the tumor staging system of Boswick *et al.* (13). Eight (65%) of 13 informative patients with locally confined prostate tumors (group A) had LOH for one or several loci within the 7q31 region. Eight (47%) of the 17 patients with extracapsular tumor extension (group B) had allelic loss in the same region, whereas LOH was observed in 4 (31%) of 13 metastatic cancers (group C). Surprisingly, the incidence of LOH in 7q31 was lower in group C (metastatic forms) than in group A (locally confined tumors).

DISCUSSION

Various approaches can be used to detect genes that may be involved in tumorigenesis when altered. Cytogenetic analyses have identified distinct chromosomal regions that might harbor such genes. Previous reports have indicated numerical chromosome 7 aberrations in prostate cancer by means of cytogenetic (17–19) or fluorescence *in situ* hybridization analysis (20, 21). At the molecular level, allelotypic studies of the long arm of chromosome 7 have revealed some cases of LOH (2, 5). We have previously described LOH of the c-*met* gene located on chromosome arm 7q (8). To determine the extent of allelic loss on 7q, we analyzed 30 clinically localized and 13 metastatic human prostate carcinomas. LOH on the long arm of chromosome 7 was found in 20 (47%) of the 43 prostate tumors studied, using 7q-specific markers. We found indications of monosomy 7 in three tumors and potential loss of the entire long arm of chromosome 7 in four tumors.

However, 13 (65%) of the 20 7q-deleted tumors showed partial (interstitial and/or telomeric) alterations on 7q. Our results pointed to a SCDR within the 7q31.1–31.2 region in 12 tumors (Fig. 1). The remaining tumor (tumor 37) showed allelic loss on 7p and a proximal loss on 7q that did not conform to the SCRD suggested above. The high rate of allelic loss on 7q suggests the presence of a novel tumor suppressor gene. Cytogenetic studies have revealed 7q deletions in other types of cancer (22–24), and LOH on 7q has been reported at the molecular level in breast cancer (25, 26), ovarian cancer (27), and stomach cancer (28).

Zenklusen *et al.* (9), using polymorphic markers on chromosome arm 7q, recently reported a high frequency of LOH in region 7q31 in clinically localized prostate tumors. Our results confirm and extend these observations, based on a larger number of localized tumors as well as metastatic forms. However,

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there are certain discrepancies between the two studies, which might be due to either a sampling bias [*e.g.*, the small number of informative cases in the study by Zenklusen *et al.* (9)] or different technical approaches. In addition, the frequencies of LOH for given loci were different except for *D7S486*. We found a lower frequency of LOH on *D7S522* (36% *versus* 83%) and a higher frequency of LOH on *D7S480* (46% *versus* 28%). In our study, 12 of 13 tumors showing partial deletion of 7q showed LOH at loci *D7S480* and/or *D7S650* and defined a SCDR more distal than that identified by Zenklusen *et al.* (9), who studied 8 tumor DNAs with partial deletions. In consequence, the deleted region within the 7q31 region is probably larger than that defined by Zenklusen *et al.* (9).

The highest rate [8 (65%) of 13] of 7q31 LOH was observed in the group of locally confined tumors, most (6/8) of which showed partial deletions involving the putative SCDR. In a previous report (8), we examined 20 locally confined prostate tumors and forms with extracapsular extension for LOH on 7q, using only the c-*met* RFLP marker, and found a frequency of 33% (3/9). Here, we also used seven microsatellites on 7q to determine whether LOH involved other loci than c-*met* in this region. LOH on at least one locus was found in 16 (53%) of the 30 clinically localized tumors. Moreover, the frequency of LOH observed on 7q31 was 62% (8/13) in group A (strictly confined tumors). This high frequency of LOH in the 7q31 region in early stage prostate cancer provides strong evidence that this region plays an important role in the early development of prostate cancer, and that inactivation of a putative tumor suppressor gene in this region is an early event in prostate tumorigenesis, as in breast cancer (29). The rates of 7q31 LOH in the locally progressive tumors [group B, 8 (47%) of 17] and forms with regional lymph node involvement or distant metastases [group C, 4 (31%) of 13] were lower than those in strictly confined tumors (group A). The difference in the LOH rate in the 7q31 region between group A (62%) and group C (31%) was not significant ($P = 0.115$) and could be due to the small number of cases studied; alternatively, a cell subclone with a 7q31 deletion in metastatic tumors (group C) could be masked by a dominant cell subclone bearing other genetic alterations specific to invasiveness. However, further investigations are required to confirm whether 7q31 deletion is less frequent in metastatic than in localized prostate tumors, because this would suggest that 7q31 deletion contributes to local growth of cancer cells and is not involved in the process by which cells acquire metastatic potential.

Finally, the rate of LOH on 7q in this study was one of the highest compared with those on other frequently deleted regions in human prostate tumors.

The high frequency of 7q LOH indicates that the long arm of chromosome 7 is important in prostate tumorigenesis. Taken together with the results of Zenklusen *et al.* (9), our data suggest that the putative tumor suppressor gene maps to the 7q31.1–31.2 region. Inactivation of such a gene in this region might occur at an early stage of prostate tumor progression and might play an important role in primary prostate tumorigenesis rather than invasive processes. Likewise, the loss of 7q in other tumors, particularly the 7q31 region in breast cancer (26), suggests that the putative tumor suppressor gene at this site may be relevant to other human cancers.

Additional studies are now required to identify the putative tumor suppressor gene affected by 7q31 LOH as well as its role in human prostate tumorigenesis.

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