

# A novel candidate tumour suppressor locus at 9q32-33 in bladder cancer: localization of the candidate region within a single 840 kb YAC

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**Loss of heterozygosity (LOH) on chromosome 9q is the most frequent genetic alteration in transitional cell carcinoma (TCC) of the bladder, implicating the presence of a tumour suppressor gene or genes on 9q. To define the location of a tumour suppressor locus on 9q in TCC, we screened 156 TCCs of the bladder and upper urinary tract by detailed deletion mapping using 31 microsatellite markers on 9q. Partial deletions of 9q were found in 10 TCCs (6%), and LOH at all informative loci on 9q was found in 77 TCCs (49%). In five low grade superficial bladder tumours, the partial deletion was localized to *D9S195* located at 9q32-33, with retention of heterozygosity at all other informative loci including *D9S103*, *D9S258*, *D9S275* and *GSN*. We constructed a yeast artificial chromosome (YAC) contig covering the deleted region in these five tumours and placed another four unmapped microsatellite markers on this contig map. Using these markers, we further defined the common deleted region to the interval between *D9S1848* and *AFMA239XA9*. The region is covered by a single YAC (852e11), whose size is estimated to be 840 kb. Our data should expedite further fine mapping and identification of the candidate tumour suppressor gene at 9q32-33.**

## INTRODUCTION

Transitional cell carcinoma (TCC) is the most common form (~90%) of malignant epithelial tumour of the bladder and upper urinary tract. Molecular genetic and cytogenetic analyses have shown that multiple genetic alterations are involved in the genesis and progression of transitional cell carcinomas. Among these alterations, loss of heterozygosity (LOH) or deletion of chromosome 9q and/or 9p is the most frequent genetic alteration (>50%) in both superficial papillary and invasive TCC (1–4). LOH studies have demonstrated the frequent occurrence of LOH at all loci on both arms of chromosome 9, and cytogenetic studies have identified frequent monosomy 9 in TCC (5–8). Detailed deletion mapping studies using microsatellite markers have defined localized deletions on the short arm and long arm of

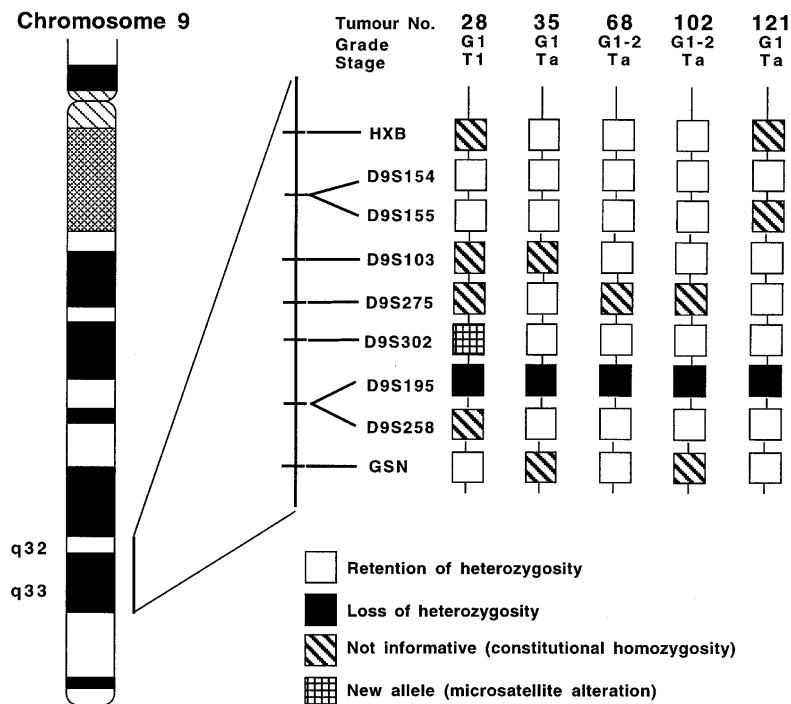
chromosome 9 (9,10). These data suggest that alterations of multiple tumour suppressor genes on chromosome 9 may occur in the genesis and progression of TCC.

Localized homozygous or hemizygous deletion at 9p21 is found in some TCCs as well as many other malignant tumours, suggesting the existence of a tumour suppressor gene at this locus (11–13). Candidate tumour suppressor genes, *p16/CDKN2/MTS1* and *p15/MTS2* identified at 9p21 have been found to be homozygously deleted in many types of human malignant tumour including TCC (14–17). On 9q, we have shown that there are at least two common deleted regions, one at 9q34 and another at 9q13-31 (18). These findings are consistent with the results of Simoneau *et al.* (19). The commonly deleted regions on 9q which have been reported to date are relatively large, and further refined localization of the candidate tumour suppressor loci has been hampered by the low frequency of partial deletions (5,6,9,10,18). However, partial deletions telomeric to 9q31 have been found in several TCCs, and the occurrence of partial deletions here is more frequent than on proximal 9q (18,19), indicating that there may be a tumour suppressor locus in this region. Furthermore, partial deletions telomeric to 9q31–32 have been reported in other types of human malignant tumour (20–26). In this study, we have attempted to detect more localized deletions in the telomeric 9q region by using a larger number of microsatellite markers. Here we report the localization of a deleted region at 9q32–33 in TCC and the construction of a yeast artificial chromosome (YAC) contig map encompassing the region. Further deletion mapping analyses suggest that the region for a candidate tumour suppressor is localized within a single YAC whose size is estimated to be 840 kb.

## RESULTS

We analysed 156 TCCs of the bladder and upper urinary tract using 31 microsatellite markers on 9q. These tumours included 26 TCCs which showed retention of heterozygosity at all informative loci examined in a previous study (10). LOH at at least one locus on 9q was detected in 87 of 156 TCCs (56%), whereas 69 (44%) showed retention of heterozygosity at all loci. Seventy seven (49%) tumours showed LOH at all informative loci on 9q. This frequency of LOH on 9q is an underestimate of the overall frequency in TCC since the present study included a selected group of 26 tumours which showed retention of heterozygosity at all informative 9q loci examined previously

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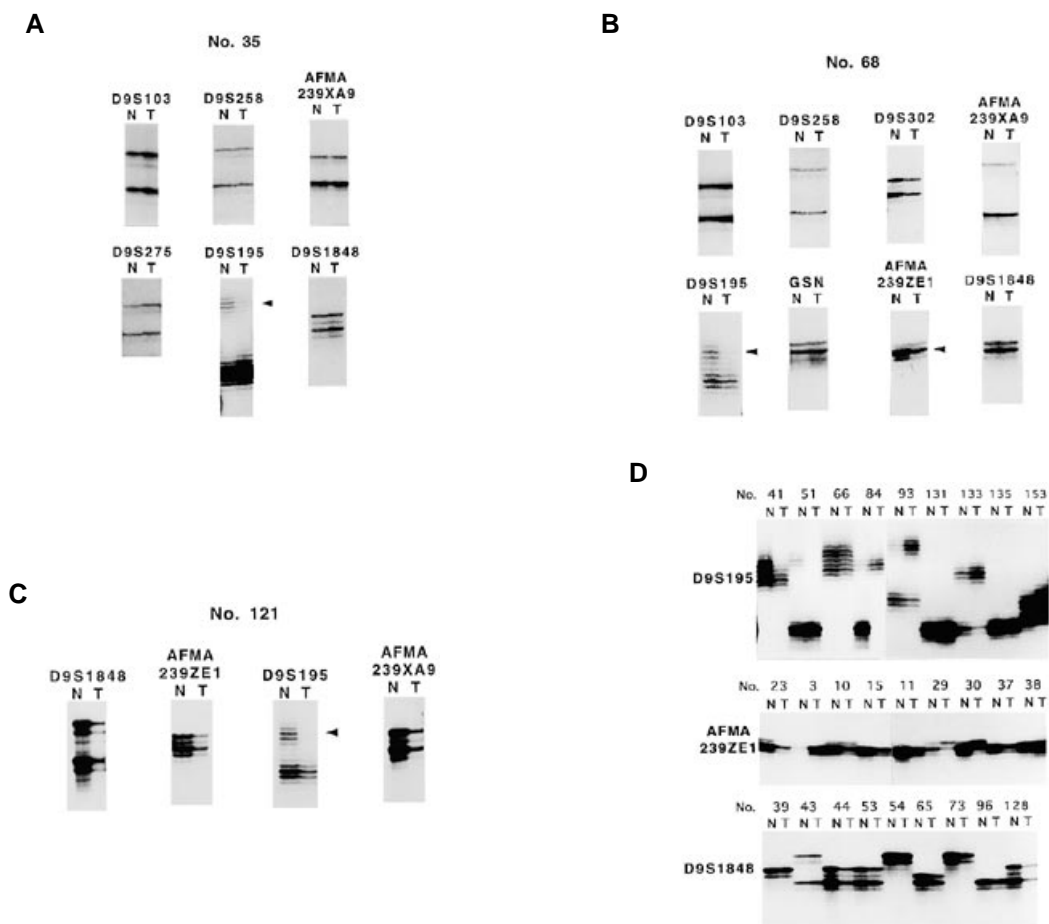
**Figure 1.** Localized deletion at 9q32–33 found in five TCCs. The five tumours depicted showed retention of heterozygosity at all other informative loci on 9q and 9p. Other examined loci are described in Materials and Methods. The order of markers from *D9S103* to *D9S195* and *D9S258* has not been defined clearly. *D9S275* has been mapped 1 cM proximal to *D9S195* and *D9S258* by Généthon.

(10). If these cases are not included, 86 of 130 TCCs (66%) showed LOH at at least one locus on 9q. Ten tumours (6%) showed partial deletions on 9q, and five of the 10 tumours (3%) had LOH at *D9S195*, which is mapped at 9q32-33, and retention of heterozygosity at all other informative loci on 9q (Figs 1 and 2). These five tumours also showed retention of heterozygosity at all informative 9p21 markers and no homozygous deletion at 9p21 by multiplex PCR analyses (17). The results for the other five partial deletions on 9q have been described previously (18), and further deletion mapping in these five tumours did not significantly narrow the localization of the deleted region (data not shown). In accordance with previous studies (1–6), the existence of LOH on 9q was not significantly associated with tumour grade and stage. LOH at at least one locus on 9q was observed in 14 of 21 grade 1 TCCs (67%), 38 of 56 grade 2 TCCs (68%) and 23 of 38 grade 3 TCCs (61%) ( $P > 0.1$ ,  $\chi^2$ ). As for stage, LOH on 9q was found in 26 of 36 Ta TCCs (72%), 16 of 31 T1 TCCs (52%), and 27 of 39 T2 or higher stage TCCs (69%) ( $P > 0.1$ ,  $\chi^2$ ). Interestingly, the five tumours with localized LOH at *D9S195* were all classified as low grade (grade 1 or 2) superficial (Ta or T1) TCCs (Fig. 1).

For the identification of a candidate tumour suppressor gene at this locus, we then constructed a YAC contig of the deleted region. The Généthon linkage map (<http://www.genethon.fr/>, March 1996) places both *D9S195* and *D9S258* 1 cM distal to *D9S275* at 135 cM from the top of chromosome 9, and other linkage data has placed *D9S195* proximal to *GSN* (27,28). However, the precise order of the markers *D9S103*, *D9S258*, *D9S275*, *D9S195*, *D9S302* and *GSN* was not defined clearly. We obtained and analysed 10 YAC clones from the Centre d'Études du Polymorphisme Humain (CEPH) (29) known to be positive for *D9S195*, *D9S258*, and *D9S275*. We also screened the ICI YAC library (30) with *D9S195*,

*D9S258*, *D9S275* and *D9S302* by PCR and we identified 11, seven and three positive clones for *D9S195*, *D9S258* and *D9S275* respectively (Fig. 3). Three CEPH YACs were found to contain *D9S103* and three other CEPH YACs contained *GSN* (Fig. 3). We could not find a YAC containing *D9S302* in the ICI library. We first constructed a YAC contig by PCR analyses using the five markers (*D9S103*, *D9S195*, *D9S258*, *D9S275* and *GSN*). To refine the YAC contig map further, we then isolated 10 YAC-end fragments from five clones by the vectorette method, and established 10 sequence-tagged sites (STSs) (Table 1). These new STSs were mapped to chromosome 9 by PCR using the human–Chinese hamster ovary hybrid cell line GM10611, which contains an intact human chromosome 9 in a Chinese hamster background (31) and the other YACs. We also identified the marker *D9S123* in CEPH YAC 765b11. Considering the linkage data and CEPH YAC data (<http://www.ceph.fr/>) indicating that YACs 755g12, 798e3, 767h1 and 765b11 overlap each other, the resulting YAC contig map is shown in Figure 3. Our data indicate the likely order of these markers as (centromere)–*D9S275*–*D9S195*–*D9S258*–*D9S103*–*GSN*–(telomere). YAC 908c11 may contain a large internal deletion. However, if we assume that no YACs have an internal deletion, the orientation of the markers from *D9S275* to *D9S103* (Fig. 3) may be reversed and the likely order is (centromere)–*D9S103*–*D9S258*–*D9S195*–*D9S275*–*GSN*–(telomere). In either case, the flanking markers for the deleted region in the five tumours with selective deletions are *D9S258* and *D9S275* (Fig. 3). Although *D9S302* previously was closely linked to *D9S195* by linkage analyses (27), this marker was absent from all the YAC clones analysed.

In order to define the deleted region further, we tried to locate other published microsatellite markers on this YAC contig map. According to Généthon linkage data and data from the Whitehead



**Figure 2.** Representative autoradiographs showing the pattern of localized deletion at 9q32–33. N, normal DNA; T, tumour DNA. Deleted alleles are indicated with arrowheads in (A), (B) and (C). (A) In tumour no. 35, LOH is found at *D9S195* with retention of heterozygosity at the other markers. (B) In tumour no. 68, LOH is observed at *D9S195* and *AFMA239ZE1* with retention of heterozygosity at the other markers. (C) In tumour no. 121, localized LOH is found at *D9S195* with retention of heterozygosity at *D9S1848*, *AFMA239ZE1* and *AFMA239XA9*. (D) Representative patterns for microsatellite markers *D9S195*, *AFMA239ZE1* and *D9S1848*. In *D9S195*, stutter (ghost) bands were observed consistently both above and below the major band representing each allele. With this marker, a significant difference in intensity between each allele is observed normally (case nos 51, 84, 93 and 35 in A) where there is large difference in allele size. Cases no. 131 and no. 135 are constitutionally homozygous (not informative) at this locus and all other tumours shown have LOH. The markers *AFMA239ZE1* and *D9S1848* show no stutter bands above the major band. Tumour no. 23 shows loss of an upper allele and tumours no. 29 and no. 30 show a clear loss of a lower allele at *AFMA239ZE1*. Note that heterozygotes for *AFMA239ZE1* show a significant difference in intensity between each allele differing only by one CA repeat unit, as seen in case no. 68 (B). At *D9S1848*, tumours nos 43, 44, 53, 65, and 128 show retention of heterozygosity and all other cases are constitutionally homozygous.

Institute–MIT Genome Center (<http://www-genome.wi.mit.edu/>, Release 11, October 1996), four microsatellite markers, *D9S1848*, *GGAA-P17524*, *AFMA239XA9* and *AFMA239ZE1* have been mapped close to *D9S195*. Using the YAC contig map, we placed *D9S1848* between 852e11-R and 9DC8-R, *AFMA239ZE1* between 814c5-L and 12IB1-R, *AFMA239XA9* between 12IB1-L and 852e11-L and *GGAA-P17524* between 15HD3-R and 814c5-R (Fig. 3). The likely order of these markers is therefore (centromere)–*D9S275*–*D9S1848*–*AFMA239ZE1*–*D9S195*–*AFMA239XA9*–*GGAA-P17524*–*D9S258*–*D9S103*–*GSN*–(telomere) (Fig. 3). Using these markers, we analysed the extent of the deletion in the five tumours with localized deletion at 9q32–33 (Figs 2 and 4). Three tumours (nos 35, 68 and 121) retained heterozygosity at *AFMA239XA9*, and all five tumours retained heterozygosity at *D9S1848*. In addition to *D9S195*, LOH was detected at *AFMA239ZE1* in tumours no. 68 and no. 102. Since only tumour no. 121 showed retention of heterozygosity at *AFMA239ZE1*, the consensus candidate region for a tumour suppressor gene is between *D9S1848* and *AFMA239XA9* (Fig. 4).

This region is covered by a single YAC (852e11) and is considered to be <840 kb, if this YAC has neither deletion nor rearrangement (Fig. 3).

## DISCUSSION

Frequent occurrence of monosomy 9 or LOH at all informative loci in TCC indicates that inactivation of multiple tumour suppressors on chromosome 9 may occur during the genesis and progression of TCCs (5–10). In support of this theory, recent studies by us and Simoneau *et al.* have shown that at least two tumour suppressor loci, one at 9q13–31 and another at 9q34, may exist on chromosome 9 in addition to that at 9p21 (18,19). In this study, we found five tumours with localized deletion at 9q32–33, indicating the presence of another candidate tumour suppressor locus for TCC in this region. Although the frequency of localized deletion at this locus is not high (3%), the presence of an important tumour suppressor gene in this region may be suggested for several reasons. First, accumulating data from

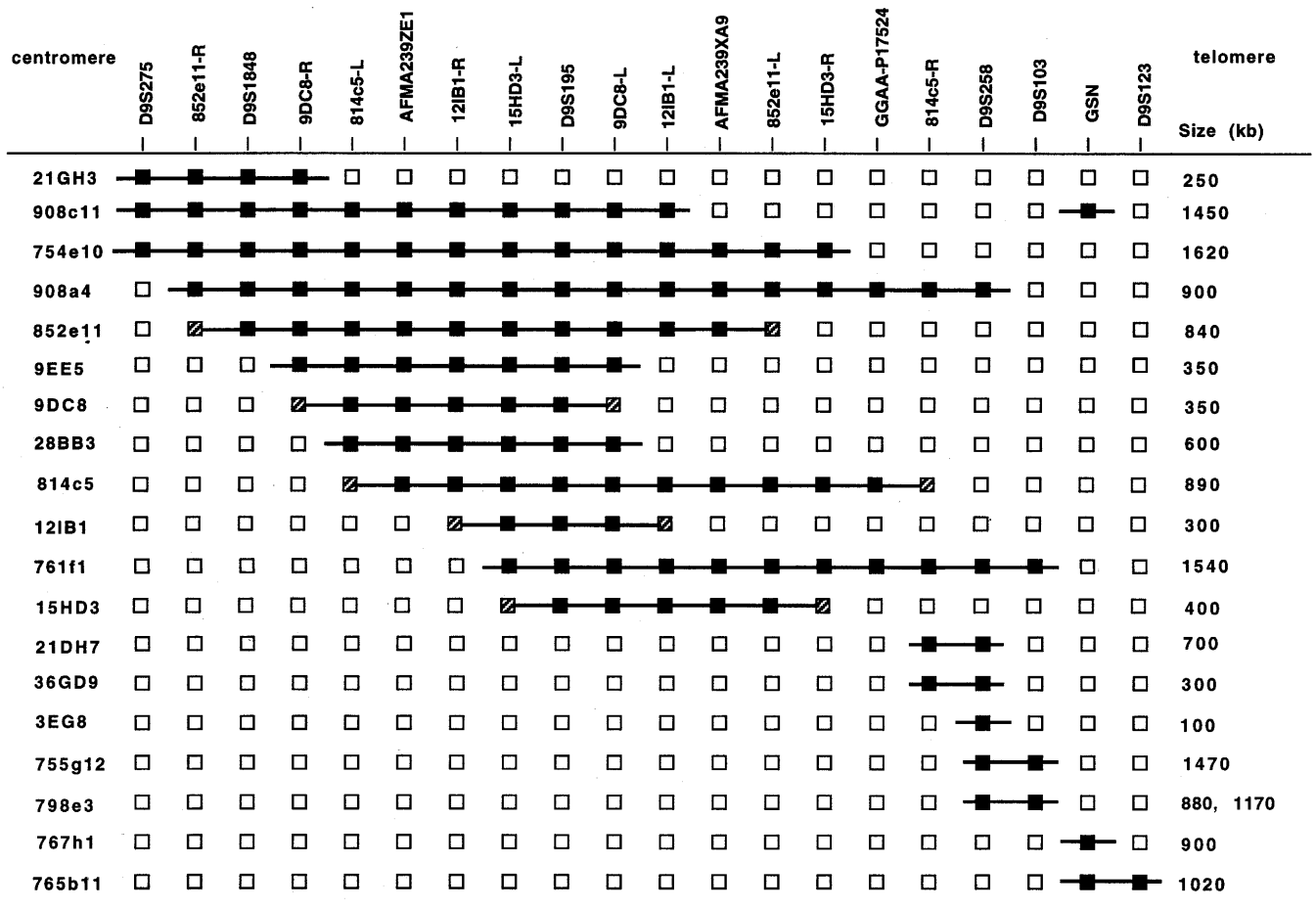


Figure 3. A YAC contig map encompassing the deleted region at 9q32-33. 3EG8, 21GH3, 36GD9, 15HD3, 12IB1, 28BB3, 9EE5, 9DC8 and 21GH3 are from the ICI YAC library (30) and other YACs are from the CEPH YAC library (29). Black and white squares indicate the presence or absence of a particular STS in each YAC, respectively. Hatched squares indicate the YAC-end STSs. -R and -L indicate right and left YAC-ends, respectively. The size of each YAC was determined by CHEF-gel electrophoresis, followed by Southern hybridization with total human DNA. The size of all CEPH YACs shown was consistent with CEPH data.

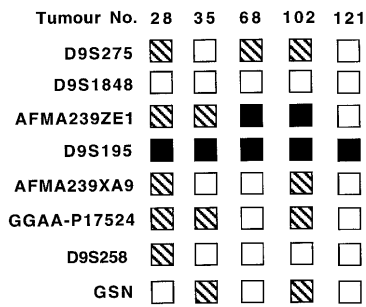


Figure 4. Further deletion mapping in the critical deleted region in five tumours with localized deletion at 9q32-33. The order of microsatellite markers was determined from the YAC contig map constructed. White, black and hatched squares indicate retention of heterozygosity, LOH and constitutional homozygote (not informative), respectively.

several deletion mapping studies in TCC have shown that partial deletions on 9q mostly encompass this region, although localized deletion at 9q34 or at 9q13-31 is also found at low frequency (5,6,9,10,18,19). So far, we have found 13 TCCs with partial deletion on 9q (10,18), and the deletion in only a single tumour does not include the region defined in this study. Second, since

LOH involving the entire long arm of chromosome 9 is found in >50% of TCCs (5,6,10,18), inactivation of a tumour suppressor at 9q32-33 and/or other tumour suppressor(s) on 9q may occur in a substantial proportion of TCCs with LOH on the whole of 9q. Furthermore, a recent study of microcell-mediated transfer of normal chromosome 9 into bladder cancer cells also indicates the presence of at least three tumour suppressor loci on 9q, and one candidate region encompasses the region defined in this study (32). Interestingly, all five TCCs with localized deletion at 9q32-33 were low grade superficial tumours (Fig. 1). Although we have not sequenced *p16* and *p15* genes in these tumours, all these tumours showed retention of heterozygosity at 9p21 and no homozygous deletion by multiplex PCR analyses (17). Therefore, it may be speculated that inactivation of a tumour suppressor located on 9q32-33 sometimes occurs in these low grade superficial tumours without inactivation of other tumour suppressors on chromosome 9. However, this is likely to be a rare event since LOH at all loci on chromosome 9 or monosomy 9 is frequently found even in low grade low stage TCCs (1-8). The identification of all relevant tumour suppressor(s) on chromosome 9 will be required to define the roles of partial or total hemizygous loss of chromosome 9 in the genesis and progression of TCC.

**Table 1.** PCR primers and conditions for new YAC-end sequence-tagged sites (STSs)

YAC-end	Primer sequence (5'-)	Size (bp)	Annealing temp. (°C)	MgCl <sub>2</sub>
814c5-L	1:GTACCTTAATAGCTACAAGAC 2:CGGAATCAATTCAGCTAAGTC	133	55	1.0
814c5-R	1:TTCATGACCCTGTACTGTTTGC 2:CACTTTGGTGATAACCTCCATTC	137	55	1.0
852e11-L	1:GAACTTACATGCCGATAGACTTTG 2:GAATTCTTGGCTCTGTACTCTGT	113	55	1.0
852e11-R	1:TGGGCTGCACAATTAGAACGTG 2:AGGGAGAATTTGACAGCGAGAT	144	55	1.0
9DC8-L	1:GCAATCAGCCTGAATGCAGGCT 2:ACTAGGGATCTGCATTGCTGAT	162	50	1.0
9DC8-R	1:GAATTCAGGAGCCATGTGGAAT 2:AGGGAAACTAGTCTCAGAGAACT	140	55	1.5
12IB1-L	1:CCATGGTTTGAAGTGCAGTGTA 2:AGGGGTATGGTCATGAAGGATA	190	55	1.0
12IB1-R	1:TTGACTGGAGAGCTAGTTTGCC 2:ACTGGATTCCTAGAGTGTATGTC	168	55	1.0
15HD3-L	1:ATCTAGCCTTGCAAAGTCTCTAC 2:AGCTTGTAACTGTAACGTGGCT	113	57	1.0
15HD3-R	1:CCTTAATGCATACAGATCAATGCC 2:AAGGACAAGTGGATCCTACCAT	81	55	1.0

LOH involving 9q has been reported in other types of human cancer, including squamous cell carcinoma of the head and neck (20,21), squamous cell carcinoma of the skin (22), ovarian cancer (23,24), renal cell carcinoma (25) and oesophageal cancer (26). The localized deleted regions reported in these other cancers encompass the region at 9q32-33 described here. One of the candidate tumour suppressor loci in ovarian cancer has been mapped between *HXB* at 9q32 and *ASS1* at 9q34.1 (23,24), which encompasses this region. Partial deletions telomeric to *HXB* and telomeric to *D9S127* at 9q22.1-32 were reported in renal cell carcinoma and head and neck squamous cell carcinoma, respectively (20,25). Although a candidate tumour suppressor locus in oesophageal cancer has been mapped at 9q31-32, which is distinct from the region reported here (26), many partial deletions in oesophageal cancer also involve the region at 9q32-33 (26). Therefore, inactivation of a candidate tumour suppressor at 9q32-33 may be involved in other types of human cancer, and further detailed deletion mapping studies in these cancers may be interesting.

As an important step for positional cloning of the tumour suppressor gene, we have constructed a YAC contig map covering the entire candidate region. The order of the microsatellite markers determined by the YAC contig map mostly conforms to existing linkage data (27,28 and Généthon) if we assume that YAC 908c11 contains an internal deletion. If this were not the case, the orientation of markers from *D9S275* to *D9S103* would be reversed (Fig. 3) and there would be a discrepancy with the existing linkage data. Such a discrepancy could be the result of a mistyping in the genotype database, or a hybrid containing an unrecognized fragment or an internal deletion within a YAC (33).

Since all 10 YAC-end STSs generated in this study have been mapped to the YAC contig and to chromosome 9, it is unlikely that these YACs are chimeric. It is suggested that the critical region for the tumour suppressor is localized between *D9S1848* and *AFMA239XA9* and is estimated to be <840 kb if the YAC 852e11 has no deletion. Since the region is also covered by six overlapping ICI YACs with relatively small size (Fig. 2), this YAC contig map will be useful for further fine mapping of this region and locating other STSs or expressed sequenced tags (ESTs).

## MATERIALS AND METHODS

### Detection of loss of heterozygosity by microsatellite analysis

Specimens of 145 TCCs of the bladder and nine TCCs of the ureter or renal pelvis were obtained with paired blood or normal kidney samples as a source of constitutional normal DNA. DNA from tumour specimens and corresponding normal tissues (peripheral blood or normal kidney) were obtained by proteinase K digestion followed by phenol/chloroform extraction. Adjacent portions of each tumour specimen were subjected to histopathological examination. Tumour stage and grade were classified according to the TNM system and the WHO criteria, respectively. We initially used 31 microsatellite markers mapped to 9q. Nine markers on 9q32-33 are shown in Figure 1. The other 22 markers on 9q used were *D9S15*, *D9S153*, *D9S167*, *D9S152*, *D9S201*, *D9S283*, *D9S119*, *D9S12*, *D9S176*, *D9S109*, *D9S127*, *D9S53*, *D9S58*, *D9S105*, *D9S59*, *D9S123*, *D9S282*, *D9S60*, *D9S61*, *ABL*,

*D9S66* and *D9S67*. To evaluate 9p status in five tumours with localized deletion at 9q32-33, we tested *D9S199*, *D9S200*, *IFNA*, *D9S1749*, *D9S126* and *D9S171* by multiplex PCR (17). Primer sequences were obtained from the Genome Database. PCR reactions were carried out in 12.5 µl reaction volumes with 5–10 ng of genomic DNA as template, 1.0–1.5 mM MgCl<sub>2</sub>, 200 µM of each deoxynucleotide triphosphate, 2 pmol of each primer, 1 U of *Taq* DNA polymerase and buffer supplied by the manufacturer (Life Technologies). One of each primer pair was end labelled with <sup>32</sup>P. PCR reactions consisted of 26–27 cycles of 1 min at 95°C, 1 min at 55°C and 1.5 min at 72°C, followed by a final elongation. Reaction products were diluted with formamide dye, heat-denatured, and run in 6% denaturing polyacrylamide gels. Gels were dried and exposed to Fuji XR film and subsequently to a PhosphorImager screen (Molecular Dynamics). Initially, LOH was screened visually for loss of one allele, and cases with 'partial loss' or 'allelic imbalance' were analysed further by the PhosphorImager using the ImageQuant software (Molecular Dynamics). A relative decrease in the intensity of the signal from one tumour allele of >40% was scored as LOH. Loci at which new alleles were detected (microsatellite alterations) were considered to be 'not informative'.

### Isolation and characterization of YAC clones

Two YAC libraries were used for YAC clone isolation and construction of a YAC contig. The ICI YAC library (30) was screened by PCR using published primer sequences for *D9S258*, *D9S275*, *D9S195* and *D9S302*. From the CEPH YAC library (29), we obtained and analysed 10 YAC clones that have been shown to be positive for one of three microsatellite markers, *D9S195*, *D9S258* and *D9S275*, or shown to be contiguous to positive YACs. High molecular weight DNA from each YAC clone was prepared in agarose blocks as described (34) and subjected to pulse-field electrophoresis in 1% agarose gels using a contour-clamped homogeneous electric field (CHEF) apparatus (BioRad- CHEF DR<sup>TM</sup> II system). Typical running conditions were as follows: 60 s pulse time for 15 h followed by a 90 s pulse time for 9 h at 200 V in 0.5× TBE buffer at 14°C. After ethidium bromide staining, gels were blotted onto nylon membranes (Hybond N+, Amersham) using 0.4 M NaOH as transfer buffer, and each blot was hybridized with total human DNA <sup>32</sup>P-labelled by random priming. The size of each YAC was evaluated by using *Saccharomyces cerevisiae* (strain YNN295, BioRad) chromosome and mutimers of λ phage (BioRad) as size markers. The size of all CEPH YACs shown in Figure 2 was consistent with CEPH data (<http://www.ceph.fr/>).

### Generation of sequence-tagged sites (STSs) from YAC-ends

STSs from YAC insert ends (YAC-ends) were generated using the modified vectorette-PCR procedure as described by Riley *et al.* (35) and direct DNA sequencing of PCR fragments. Briefly, YAC DNAs were prepared as described (34) and three vectorette libraries were prepared for each YAC. YAC DNA was digested with *Rsa*I, *Alu*I or *Pvu*II, and then ligated with the blunt-end vectorette cassette as described (35). Using these three vectorette libraries as templates, PCR was performed with 224 primer (35) and a primer 5'-CTACTTGGAGCCACTATCGACTACGC-GATC to isolate the left arm of each YAC and with 224 primer

and a primer 5'-CTTGCAAGTCTGGGAAGTGAATGGAGACAT to isolate the right arm. The resulting PCR products were electrophoresed in agarose gels, and amplified fragments of appropriate size were recovered and used as sequencing templates. Direct sequencing was performed using internal primers 1207 and 368 (35) for the left YAC-end fragments and 1208 and 368 (35) for the right YAC-end fragment using a cycle sequencing kit (*fmol* sequencing system, Promega). Oligonucleotides which can amplify a 81–190 bp PCR fragment from each YAC-end sequence were generated (Table 1). The presence or absence of these STSs in each YAC clone was tested at least twice by PCR amplification and agarose gel electrophoresis. DNA from a hybrid cell line GM10611 containing an intact human chromosome 9 in a Chinese hamster background (31) and original YAC clone DNAs were used as positive control templates, and normal hamster fibroblast DNA as a negative control template.

### Construction of the YAC contig map

Using microsatellite markers and 10 new YAC-end STSs, a YAC contig map was constructed by PCR-based analyses. PCR reactions were performed with 20 ng of YAC DNA or yeast cell pellets washed with 1× TE in 25 µl reaction volumes using 1 U of *Taq* DNA polymerase with 200 µM concentrations of each dNTP and 1.0–1.5 mM of MgCl<sub>2</sub>. PCR reactions were carried out routinely using a 'hot-start', in which *Taq* polymerase was added to the reaction after a 5 min denaturing step at 95°C. Thirty to 35 amplification cycles with 95°C for 60s, 50–55°C for 60s and 72°C for 90s were performed.

### Uniform resource locators (URLs) for public genome data

We used the following publicly available data from the World Wide Web.

The Centre d'Etudes du Polymorphisme Humain (CEPH):

<http://www.ceph.fr/>

Généthon: <http://www.genethon.fr/>

The Genome Database: <http://gdbwww.gdb.org/gdb>

Whitehead Institute for Biomedical Research/MIT Center for Genome Research: <http://www.genome.wi.mit.edu/>

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