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Original Paper

Molecular Genetic Analysis of the Von Hippel–Lindau Disease (VHL) Tumour Suppressor Gene in Gonadal Tumours

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Chromosome 3p allele loss is frequent in ovarian and testicular tumours. The von Hippel–Lindau (VHL) disease tumour suppressor gene maps to chromosome 3p25. Gonadal tumours may occur in patients with VHL disease, so somatic *VHL* gene mutations might be involved in the pathogenesis of sporadic gonadal tumours. To investigate this hypothesis, we screened 60 gonadal tumours (36 ovarian and 24 testicular) for *VHL* gene mutations and chromosome 3p allele loss. Although 38% (10/26) of informative ovarian and 54% (7/13) of testicular tumours demonstrated 3p allele loss, no somatic *VHL* gene mutations were detected in the 60 gonadal tumours analysed. This suggested that chromosome 3p tumour suppressor gene(s) other than *VHL* are involved in gonadal tumorigenesis.

Key words: von Hippel–Lindau, VHL, chromosome 3p, ovarian cancer, testicular cancer
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INTRODUCTION

CYTOGENETIC AND molecular studies have revealed deletions or allele loss on the short arm of chromosome 3 in cancers of the lung, kidney, breast, ovary and testicle [1]. Detailed deletion mapping studies in renal and lung carcinomas have provided evidence for multiple tumour suppressor genes on the short arm of chromosome 3 [2–7]. The interpretation of loss of heterozygosity (LOH) studies can be difficult and the detection of somatic mutations in a candidate tumour suppressor gene provides more direct evidence for a role in carcinogenesis. The first tumour suppressor gene isolated from the short arm of chromosome 3 is the von Hippel–Lindau (*VHL*) disease gene which maps to 3p25–p26 [8]. Germline *VHL* gene mutations predispose to a variety of tumours, most commonly retinal and cerebellar haemangioblastomas, renal cell carcinoma and phaeochromocytoma [9]. In addition, testicular tumours may occur occasionally in VHL patients ([10] and unpublished observations). Tumours from VHL patients frequently show loss of the wild type allele, so that the mechanism of tumorigenesis appears similar to that in inherited retinoblastoma [11]. Furthermore, somatic *VHL* gene mutations can be detected in approximately 50% of non-familial clear cell renal carcinomas, and most tumours with *VHL* mutations have a chromosome 3p deletion including the other *VHL* allele [12–15]. Somatic *VHL* gene mutations have also been reported in non-familial cerebellar haemangioblastomas [16]. These findings are compatible with the Knudson model of tumorigenesis, similar to the role of *RB1* mutations in sporadic retinoblastoma.

While germline mutations in a tumour suppressor gene may predispose to familial cancer syndromes with a very specific phenotype, for some genes such as *RB1* and *NF1*, somatic mutations may be detected in a much wider spectrum of cancers [17]. Therefore, somatic *VHL* mutations might contribute to carcinogenesis in tumours showing 3p allele loss even if these tumour types are not seen in patients with VHL disease. Chromosome 3p allele loss has been reported in up to 50% of ovarian cancers [18–20] and 46% of testicular tumours [21]. To investigate the potential role of the *VHL* gene in the pathogenesis of gonadal tumours, we analysed 36 ovarian and 24 testicular tumours for somatic *VHL* gene mutations.

MATERIALS AND METHODS

Patient and tumour material

Paired blood and tumour samples were obtained from 36 women with ovarian cancer and 24 men with testicular tumours. Most tumour samples were taken from primary tumours in

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previously untreated patients, and were snap frozen in liquid nitrogen and stored at −30 or −70°C until analysed. However, four testicular teratomas were secondary tumours after chemotherapy and a small number of ovarian tumours were from ascites.

Molecular genetic analysis

High molecular weight DNA was isolated from peripheral blood and frozen tumour tissue by standard methods as described previously [20, 22]. The cloned coding sequence (852 bp) of the *VHL* disease gene is represented in three exons, and this was amplified using five PCR primer sets, with SSCP analysis performed according to our previously published protocol [12, 23]. The nucleotide sequence of any PCR products showing bandshifts on SSCP analysis was to be determined by direct sequencing with commercially available cycle sequencing kit (Gibco BRL), according to the manufacturer's guidelines.

A limited examination for chromosome 3p allele loss was performed with microsatellite markers at D3S1038, D3S1067 and D3S1076 as described previously [6]. In addition, 18 ovarian cancers were also analysed for LOH using a diallelic polymorphism at THRB [24].

RESULTS

Ovarian cancer

Molecular genetic analysis for chromosome 3p allele loss was performed using polymorphisms at D3S1038, D3S1067, D3S1076 and THRB. The regional localisations of these loci are shown in Figure 1. D3S1038 maps within 500 kb centromeric of the *VHL* gene. Twenty six of the 36 tumours analysed were informative at one or more loci, and 10 tumours (38% of informative cancers) showed LOH at one or more of the loci examined (Table 1 and Figure 2). Ovarian cancers with and without 3p LOH were compared for tumour stage and histopathology, but no differences were identified between the two groups (Table 1). Similarly there was no difference in mean age at diagnosis (data not shown). Analysis of the pattern of LOH in ovarian cancer No. 12 suggested that a critical region for allele loss maps centromeric to D3S1038 and the *VHL* gene.

Screening for intragenic *VHL* gene mutations by SSCP analysis showed no abnormality in any of the 36 cancers examined (Figure 3).

Testicular cancer

The 15 tumours for which constitutional DNA was available were analysed for LOH at D3S1038, D3S1067 and D3S1076. Thirteen tumours were informative at one or more loci, and seven of these (54% of informative tumours) demonstrated LOH at one or more loci (Table 2). No significant differences in histopathology or age at operation were detected between those tumours with and without 3p LOH.

All 24 testicular tumours were screened for intragenic *VHL* gene mutations by SSCP analysis but no abnormality was detected in any tumour.

DISCUSSION

This analysis of 36 ovarian tumours for LOH on chromosome 3p represents the largest study yet performed and confirms the results of previous smaller studies [18, 19], that chromosome 3p allele loss is frequent in ovarian cancer. We also confirmed the findings of Murty and associates [21] that chromosome 3p allele loss is frequent in testicular tumours. However, our failure to detect somatic *VHL* mutations in 60 gonadal (36 ovarian and 24 testicular) tumours suggests that the *VHL* gene is not the principal target of the chromosome 3p LOH detected, and that somatic *VHL* gene mutations do not have a significant role in the pathogenesis of gonadal tumours. Although SSCP analysis will not detect all *VHL* gene mutations, we have identified somatic *VHL* mutations in sporadic renal cell carcinomas [12] using similar techniques. The incidence of somatic *VHL* gene mutations in renal cell carcinomas with 3p LOH is significantly higher than in gonadal tumours with 3p allele loss (0/17 versus 30/65, $\chi^2 = 9.83, P < 0.01$). In addition, Gnarra and associates [13] and Whaley and associates [15] did not detect somatic *VHL* mutations in a total of 16 ovarian cancers examined. Although

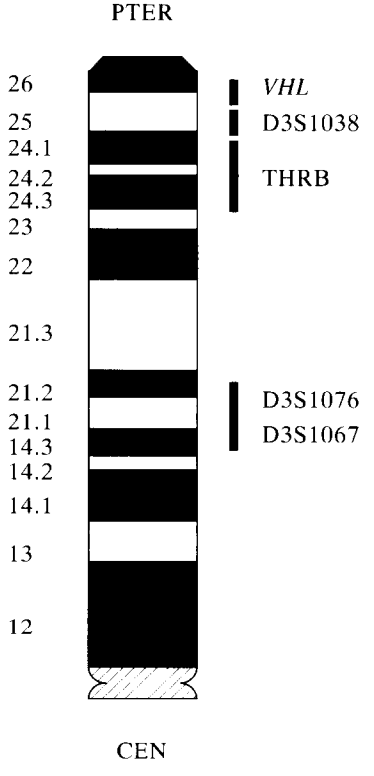

Figure 1. Ideogram of the short arm of chromosome 3 showing location of D3S1038, THRB, D3S1067 and D3S1076 loci.

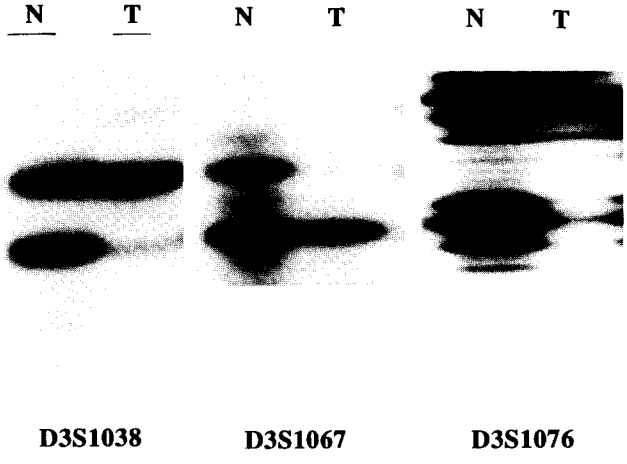

Figure 2. Loss of heterozygosity in ovarian cancer (tumour 52) at D3S1038, D3S1067 and D3S1076. N, blood DNA, T, tumour.

Table 1. Clinical details and results of chromosome 3p allele loss studies in 26 informative ovarian tumours

Tumour ID	Histology	Grade	Stage	D3S1038	THRB	D3S1076	D3S1067
11	Serous	PD	II	—	—	LOSS	—
12	Serous	MD	I	RET	LOSS	RET	RET
16	Adenocarcinoma	PD	III	LOSS	—	RET	—
52	Adenocarcinoma	PD	III	LOSS	—	LOSS	LOSS
62	Adenocarcinoma	—	III	LOSS	LOSS	—	RET
63	Serous	PD	III	LOSS	—	LOSS	—
77	Endometroid	—	III	LOSS	—	RET	RET
78	Adenocarcinoma	—	III	—	—	LOSS	RET
95	Adenocarcinoma	—	III	LOSS	—	—	—
122	Adenocarcinoma	—	III	LOSS	—	RET	RET
3	Serous	WD	III	RET	—	RET	RET
10	Serous	—	III	RET	—	RET	RET
13	Serous	PD	III	—	RET	RET	RET
30	Serous	MD	III	RET	—	RET	RET
40	Clear cell	MD	III	RET	—	—	—
47	Serous	—	III	RET	—	—	RET
49	Adenocarcinoma	PD	III	RET	RET	—	—
51	Serous	PD	III	—	RET	—	—
54	Endometroid	PD	III	RET	—	RET	RET
55	Adenocarcinoma	PD	III	—	RET	—	—
56	Serous	MD	III	—	RET	RET	—
68	Serous	MD	III	RET	—	—	RET
76	Serous	PD	III	RET	—	RET	RET
79	Adenocarcinoma	—	III	RET	—	RET	—
92	Serous	MD	III	—	—	RET	—
94	Serous	PD	III	—	—	RET	RET

WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated; —, not specified; RET, retention of heterozygosity at this locus; LOSS, loss of heterozygosity (LOH) detected.

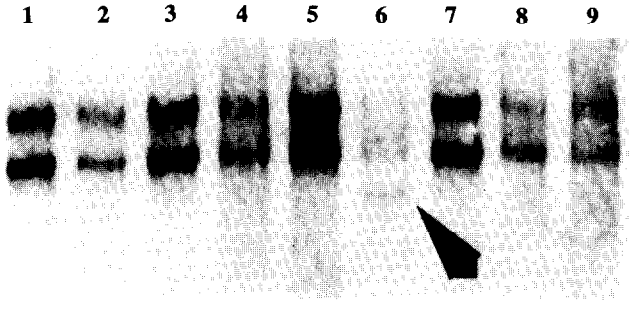

Figure 3. Example of SSCP analysis for exon 3 of the *VHL* tumour suppressor gene. Lanes 1 to 5, ovarian cancer DNA (tumours 3, 8, 10, 11 and 12); lane 6, positive control; lanes 7 to 9, ovarian cancer DNA (tumours 13, 30 and 39).

chromosome 3p allele loss is common in lung cancer, Sekido and associates [25] found somatic missense *VHL* mutations in only two of 72 cell lines examined.

Chromosome 3p allele loss is frequent in ovarian cancer, but many other regions of the genome show a significant incidence of LOH including 5q, 6p, 6q, 7p, 8p, 9q, 13q, 14q, 17p, 17q, 18q and 22q [20, 26]). Similarly, multiple mutational events, including allele loss at chromosome 3p, 9p, 9q, 10q, 11p, 11q, 12q, 13q, 17p, 17q and 18q have been reported in testicular tumours [21]. However, amplification of chromosome 12p, frequently the formation of isochromosome 12p, appears to be

Table 2. Clinical details and results of chromosome 3p allele loss studies in 11 informative testicular tumours

Tumour number	Histology	Age at surgery	D3S1038	D3S1076	D3S1067
1	SEM	46	RET	—	LOSS
4	SEM/MTU	38	LOSS	LOSS	LOSS
5	SEM	46	RET	RET	LOSS
6	MTI	24	LOSS	LOSS	LOSS
8	SEM	30	LOSS	—	LOSS
10	MTI/SEM	33	—	—	LOSS
17	TD	16	—	—	LOSS
3	SEM	45	RET	RET	RET
9	TD	31	RET	RET	RET
13	TD	32	RET	—	—
15	SEM/TD	35	RET	—	—
16	SEM/MTU	37	RET	—	—
18	SEM	32	—	RET	RET

TD, teratoma differentiated; MTI, malignant teratoma intermediate; MTU, malignant teratoma undifferentiated; SEM, seminoma; RET, retention of heteroxygosity at this locus; —, uninformative; LOSS, loss of heterozygosity (LOH) detected.

an early and specific event in testicular germ cell tumorigenesis [27]. It is not clear hether 3p allele loss is an early or late event in ovarian cancer, and we did not find any correlation with tumour stage or histopathological classification. Nevertheless, the suppression of tumorigenesis in an ovarian tumour cell line

by the introduction of a normal chromosome 3 suggests that 3p deletions play a crucial role in ovarian carcinogenesis [28]. The absence of *VHL* gene mutations and the interstitial LOH observed in ovarian tumour 12, which did not include the *VHL* region, suggests that other 3p tumour suppressor genes are relevant. The detection of homozygous deletions at 3p12–p13 and 3p21 in small cell lung cancers [4, 7], and detailed mapping of the multiple regions of 3p allele loss in sporadic renal cell carcinoma [2, 6] are consistent with two or more tumour suppressor genes mapping centromeric to the *VHL* gene. Although detailed information on the critical regions of chromosome 3p LOH in ovarian cancer has not been reported previously, Rimessi and associates [28] reported that three regions of chromosome 3p (two in 3p21.1–p21.2 and one in 3p23–p24) may produce tumour suppressor activity in ovarian carcinoma cell lines. These results would be compatible with the LOH results in ovarian tumour 12. *hMLH1*, a DNA mismatch repair gene, maps to chromosome 3p21. Germline *hMLH1* mutations are associated with hereditary non-polyposis colon cancer syndrome, and ovarian carcinoma may occur in this disorder [29, 30]. Somatic mutations in *hMLH1* could be involved in the pathogenesis of ovarian cancer. However, we did not detect microsatellite instability in any of the ovarian tumours we analysed. The identification of new chromosome 3p tumour suppressor genes and mutation analysis of *hMLH1* and other candidate genes should clarify the role of chromosome 3p genes in gonadal carcinogenesis.

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