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Short Communication

Loss of Heterozygosity at the *DCC* Gene Locus is Not Crucial for the Acquisition of Metastatic Potential in Oesophageal Squamous Cell Carcinoma

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Tumour specimens from 111 patients with oesophageal squamous cell carcinoma were screened for loss of heterozygosity (LOH) at the deleted colorectal carcinoma (*DCC*) gene locus. *DCC*-LOH ocurred in 10 of 61 informative cases (16%). No statistically significant correlation was observed between *DCC*-LOH and lymph node metastasis, histopathological grade or tumour stage. The survival of patients exhibiting *DCC*-LOH was not statistically different from that of patients without LOH. These results suggest that LOH at the *DCC* locus is not related to the acquisition of metastatic potential or the state of tumour cell differentiation in oesophageal squamous cell carcinoma.

Key words: *DCC*, oesophagus, squamous cell carcinoma, lymph node metastasis, prognosis, tumour suppressor gene

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INTRODUCTION

METASTASES ARE thought to develop partly as a result of the disruption of the cell adhesion system. The amino acid sequence of the deleted colorectal carcinoma (*DCC*) gene, a putative tumour suppressor gene, demonstrates a large degree of homology with the neural cell adhesion molecule (N-CAM) and other related cell surface glycoproteins of the immunoglobulin superfamily [1]. Allelic deletion at the *DCC* locus occurs more frequently in metastases of colorectal carcinoma than in primaries [2]. In addition, the expression of *DCC* mRNA, as detected by the reverse transcriptase polymerase chain reaction (RT-PCR), is lower in primaries and liver metastases of colorectal carcinomas than in adjacent non-cancerous tissues [3]. This led us to hypothesise that disruptions of the *DCC* gene might contribute to the acquisition of metastatic potential and therefore might be a good prognostic indicator.

Deletions of the *DCC* locus have been reported in 20–24% [4–6] of oesophageal squamous cell carcinomas (OSCCs), and point mutations in 4% [4]. However, the contribution of alterations in the *DCC* gene to the development of metastatic potential is controversial [4–6]. In addition, the number of subjects in the reported studies have been too small to evaluate statistically the prognostic significance of loss of heterozygosity at the *DCC* locus (*DCC*-LOH) [4–6].

To clarify the association between *DCC*-LOH and lymph node metastases or disease outcome in OSCC, we screened 111 patients with OSCC who had undergone potentially curative resections for *DCC*-LOH. We chose PCR (polymerase chain reaction) to assay for LOH [5] using two types of polymorphic markers within the *DCC* gene, namely variable number of tandem repeat (VNTR) and restriction fragment length polymorphism (RFLP), because the procedure is simple and rapid, and therefore easily transferrable to routine clinical use.

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MATERIALS AND METHODS

Tissues and DNA extraction

One hundred and eleven specimens of OSCC were obtained from patients who had received a curative oesophagectomy from 1989 to 1994 and were followed for 3–69 months, with a median of 35 months. Clinical staging and histopathological classification were determined by the TNM staging system. The tumours and normal tissues were frozen immediately after surgery, and stored at −80°C until use. DNA was extracted conventionally by proteinase K digestion and phenol–chloroform extraction. We were able to extract DNA from both primaries and their metastatic tumours in nine of the 61 tumours (15%) with lymph node metastases. DNA of metastatic lesions was extracted from microdissected materials, which had been fixed and embedded by the AMeX method [7], as previously described [8]. We confirmed microscopically that all metastatic lesions contained less than 10% normal cells.

PCR-LOH assay at the DCC *locus*

PCR was performed on the two polymorphic sites within the *DCC* gene under conditions previously described [5]. The primer sequences for amplification of the VNTR site were 5′-GATGACATTTTCCCTCTAG-3′ and 5′-GAGGTTATTGCCTTGAAAAG-3′. Those for the RFLP site were 5′-TGCACCATGCTGAAGATTGT-3′ and 5′-AGTACAACACAAGGTATGTG-3′. PCR products containing the RFLP sequence were digested with *Msp*I prior to electrophoresis. The PCR products were electrophoresed directly on a 3% agarose gel and stained with ethidium bromide. LOH was defined as a visible change in the allele:allele ratio in the tumour DNA relative to the ratio in normal DNA.

Statistics

The statistical significance of differences was evaluated by Kruskal–Wallis test with a criterion of $P<0.05$. Survival curves were plotted using the Kaplan–Meier method, and statistical significance was calculated by the generalised Wilcoxon test.

RESULTS

Of the 111 cases examined, there were 53 (48%) informative cases at the VNTR site and 41 (37%) at the RFLP site (Figure 1). *DCC*-LOH was confirmed in 9 of the 53 (17%) informative cases at the VNTR site and in 7 (17%) of the 41 informative cases at the RFLP site. Therefore, LOH at the *DCC* locus was found in a total of 16% (10/61) of the informative subjects. Five of the nine tumours, in which we were able to obtain DNA from the metastasis, were informative concerning *DCC*-LOH. Four tumours exhibited *DCC*-LOH and the remaining one retained heterozygosity. In 1 (case 97) of the 4 cases exhibiting *DCC*-LOH at the primary site, *DCC*-LOH was not detected in the metastatic site (Figure 1). There was no statistically significant correlation between *DCC*-LOH and the presence of lymph node metastases, tumour grade or tumour stage (Table 1). The survival rate of patients exhibiting *DCC*-LOH was not statistically different from that of patients without LOH.

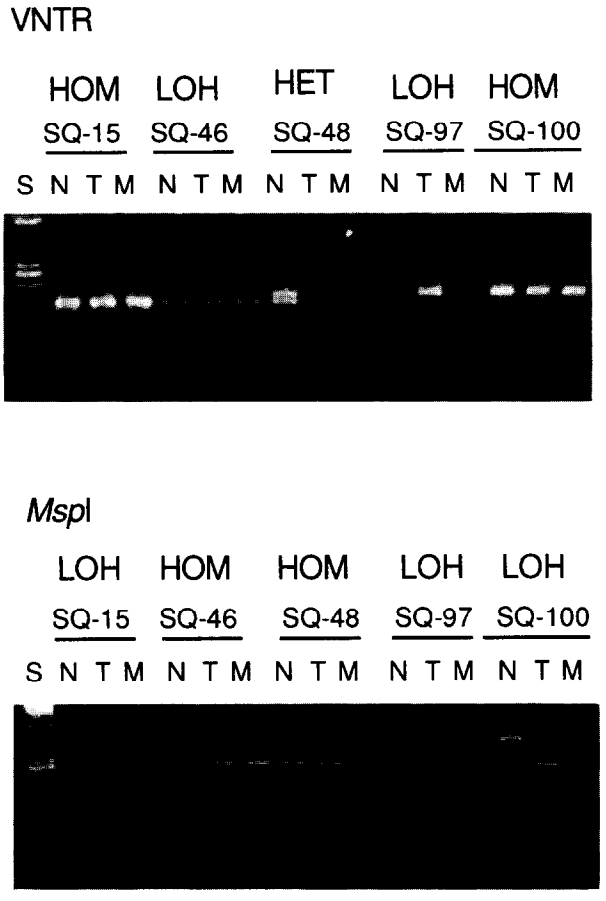

Figure 1. Representative illustrations of the PCR-LOH assay at VNTR and *Msp*I sites within the *DCC* gene in tumours with metastases. S, size marker; N, DNA extracted from normal cells; T, DNA extracted from tumour cells in primary site; M, DNA extracted from tumour cells in metastatic lesion; LOH, loss of heterozygosity; HET, retained heterozygosity; HOM, homozygosity. Loss of the upper band at the VNTR site is obvious in both T and M of SQ-46, and the lower band is lost in T but not in M of SQ-97. Loss of the uncut band at *Msp*I site is obvious in both T and M of SQ-15 and SQ-100, and the uncut band is lost in T but not in M of SQ-97.

DISCUSSION

The incidence of *DCC*-LOH presented in this study is concordant with three previous reports [4–6], although the relationship between *DCC*-LOH and metastatic potential varies among the different studies. Miyake and associates [4] have asserted that alterations in the *DCC* gene increased the metastatic potential of OSCC, because the frequency of *DCC*-LOH increased in tumours with distant lymph node metastases. In addition, they found LOH only in moderately or poorly differentiated types of OSCC and not those that were well differentiated. Therefore, *DCC*-LOH was associated with the degree of differentiation [4]. The other two reports found no significant correlation between *DCC*-LOH and tumour grade or lymph node metastases [5, 6]. If *DCC*-LOH is crucial for the acquisition of metastatic potential, the frequency of LOH should increase as the clinical stage progresses, and LOH should be detected more frequently in metastases than in primaries. In colorectal carcinoma, Ookawa and associates [2] demonstrated that LOH at the *DCC* locus was detected in all liver metastases (19/19) and in 75% (18/24) of the primary tumours. The incidence of LOH was significantly higher in Dukes' stage D tumours than in stage A, B or C tumours [2]. We demonstrated no significant correlation between *DCC*-LOH and lymph node metastases, tumour grade or tumour stage in OSCC, although there was a tendency toward more frequent *DCC*-LOH in patients with lymph node metastases.

Table 1. *Correlation of* DCC-*LOH with clinicopathological characteristics*

Clinicopathological characteristics	No. of patients (%) *DCC*-LOH(+)	*DCC*-LOH(−)	*P* value
Stage			
0	0 (0)	1 (100)	
I	1 (13)	7 (88)	
IIA	1 (8)	12 (92)	$P = 0.648$
IIB	4 (29)	10 (71)	
III	4 (16)	21 (84)	
Grade			
Well	4 (14)	14 (76)	
Moderate	4 (14)	24 (86)	$P = 0.730$
Poor	2 (13)	13 (87)	
Lymph node metastases			
Present	8 (21)	31 (79)	$P = 0.251$
Absent	2 (9)	20 (91)	

In addition, there was 1 case in which *DCC*-LOH was detected in the primary tumour but not in the metastatic lymph node, although tumour cells were carefully microdissected from normal lymphocytes. This suggests that a clone from the primary tumour became metastatic before LOH occurred in the primary tumour, or that the metastasis arose from a completely separate (synchronous or metachronous) primary tumour, which was not analysed.

Shibagaki and associates [6] have demonstrated that the commonly deleted region of chromosome 18q in OSCC did not include the *DCC* locus. They suggested that a possible tumour suppressor gene on 18q other than the *DCC* gene was involved in OSCC.

Our trial of screening for *DCC*-LOH as a prognostic indicated failed in OSCC. There are several candidate prognostic markers in OSCC that act as suppressors of invasion. Reductions in E-cadherin and α-catenin expression correlate with the presence of lymph node metastasis [9]. Deletions of the 3p25 locus, the location of the von Hippel–Lindau disease gene, is frequently detected in OSCC with lymph node metastases [10]. Knowledge of these and/or other unknown factors relating to cell adhesion systems may improve the prognostic evaluation of patients with OSCC.

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