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Advances in Brief

Tumor Progression and Loss of Heterozygosity at 5q and 18q in Non-Small Cell Lung Cancer[1]

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Abstract

We investigated the frequency and clinical significance of loss of heterozygosity (LOH) at the *APC*, *MCC*, and *DCC* tumor suppressor gene loci in 108 cases of resected non-small cell lung cancer (NSCLC). LOH at the *APC/MCC* gene cluster at chromosome 5q21 occurred frequently; it affected 29% of informative NSCLC cases and correlated with a significantly worse survival ($P < 0.01$). Furthermore, in the subtype most frequently affected (SCC), LOH at 5q not only correlated with a worse survival but also tumor involvement of the mediastinal and/or hilar nodes. In contrast, LOH at the *DCC* locus at chromosome 18q was far less frequent, occurring in 14% of NSCLC cases, and it was not associated with advanced stage or prognosis. These data suggest that LOH at 5q has a role in determining tumor progression and survival in NSCLC, and may prove to be a clinically useful prognostic indicator.

Introduction

Lung cancers are characterized by multiple genetic changes which include the activation of proto-oncogenes and the inactivation of tumor suppressor genes (1). Common mechanisms that contribute to the inactivation of a tumor suppressor gene include mutation and allelic deletion, the latter being indicated by the development of LOH[4] in tumor tissue. In the most common lung cancer type (NSCLC), the chromosomes most frequently affected by LOH include 1q, 2q, 3p, 5q, 8q, 9p, 13q, and 17p (1, 2).

On chromosome 5, the major region deleted in NSCLC maps to within a few megabases of the *APC* locus at 5q21 (3). The *APC* gene locus is closely linked to the *MCC* gene (4–7). Both genes are thought to be important candidate tumor suppressor genes which are inactivated at an early stage of colon carcinogenesis (8, 9). The *DCC* gene is also a candidate tumor suppressor gene, which is situated on chromosome 18q and seems to play a later role in the malignant transformation of colorectal cells (10).

Apart from colorectal cancers, chromosome 5q21 deletion also occurs in a number of other solid tumors including the two major types of lung cancer, NSCLC and SCLC (2, 3, 11, 12). In NSCLC, deletion of 5q21 has been reported to be far more common in advanced (70%) (3) than in early stage resected cancers (20–25%) (12, 13). These data have thus led to speculation that 5q21 loss may play a role in the progression of lung cancer.

To investigate this possibility, we studied 108 fully staged, resected NSCLC for the frequency and clinical significance of LOH at the *APC* and *MCC* loci on 5q21 in comparison to LOH at the *DCC* locus on 18q. LOH at the *APC/MCC* cluster occurred in 29% of informative NSCLC cases and was associated with a worse outcome. In the SCC subtype, LOH at 5q21 also correlated with tumor involvement of the regional lymph nodes. Conversely, LOH at the *DCC* locus at 18q was infrequent (14%) and was not associated with adverse clinical features.

Materials and Methods

Samples. The study population consisted of 108 cases of resected NSCLC from The Prince Charles Hospital (Brisbane, Australia). There were 76 males and 32 females with an age range of 28–81 years (mean age, 61 years at diagnosis). Selected parts of tumor and normal lung tissue were rapidly frozen in liquid nitrogen, and high molecular weight DNA was extracted by standard techniques. Independent histological examination of the tumors was performed according to 1982 WHO criteria and pathologically confirmed pTNM stage was assigned in accordance with the International Union Against Cancer.

Loss of Heterozygosity. LOH was detected on the basis of PCR amplification of polymorphisms within the *MCC*, *APC*, and *DCC* genes. PCR of the *MCC* gene using exon 10 primers amplified alleles of either 79 or 93 base pairs due to an insertional/deletional polymorphism (14). PCR of *APC* gene exon 11 amplified a 133-base pair target sequence containing a polymorphic *Rsa*I restriction site with restriction fragments of 87 and 46 base pairs (15). PCR mixtures were 40 and 30 μl total volume for the *MCC* and *APC* genes, respectively, containing 100 ng DNA, 1 μM of each primer, 1× Promega PCR buffer, 1.5 mM $MgCl_2$, 250 μM dNTPs, and 2.5% formamide. Promega Taq polymerase (1 and 0.75 units) was used for the *MCC* and *APC* PCR, respectively. A *DCC* dinucleotide microsatellite polymorphism (16) was examined using a similar PCR mix except that 2.4 μCi [^{35}S]dCTP was substituted for dCTP and 0.2 μM of each primer was used in a total volume of 20 μl. The cycling conditions for the *MCC* and *DCC* PCR were initial denaturation at 94°C for 3 min, 30 cycles of 92°C denaturation for 45 s, 55°C annealing for 90 s, and elongation at 72°C for 90 s. Conditions for the *APC* PCR were initial denaturation at 95°C for 5 min, then 30 cycles of 94°C denaturation for 30 s, 60°C annealing for 60 s, and 72°C extension for 90 s. Final extension for all PCR reactions was 72°C for 7 min. The *MCC* and *APC* (after digestion of 10 μl of product with 10 units of *Rsa*I) PCR products from corresponding tumor and normal lung were visualized by ethidium bromide staining after electrophoresis on 12% polyacrylamide with 10% glycerol gels at 200 V for 45 min (Mini-Protean, BioRad). The radiolabeled *DCC* microsatellite products were electrophoresed on denaturing 5% polyacrylamide gels and visualized after autoradiography with Kodak XAR film. Negative controls were incorporated with each PCR run and LOH was scored independently by two observers. Abnormal samples were repeatedly tested in independent PCR reactions and separate gel loadings to ensure reproducibility.

Statistics. Statistical analysis was performed using χ^2 and Fisher's exact test for differences between groups. Actuarial survival curves were examined using Kaplan-Meier log rank testing with survival calculated from the day of histological diagnosis. The mean and maximum follow-up duration of the study population at time of analysis was 20 and 42 months, respectively.

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[4] The abbreviations used are: LOH, loss of heterozygosity; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.

Table 1 *The clinical features of NSCLC with and without LOH at 5q and 18q*

	Cases with LOH at 5q	Informative cases without LOH		Cases with LOH at 18q	Informative cases without LOH	
Sex						
Male	18	33		8	55	
Female	4	20	NS,[a] χ^2	5	22	NS, χ^2
Mean age (yr)	59.2	61.7		62.5	60.7	
95% confidence level	5.5	2.9		3.6	2.6	
Histological subtype						
SCC	12	20		6	31	
AC	4	24	$P = 0.04$, Fisher's test	5	30	NS, χ^2
Large cell	2	2		1	3	
Adenosquamous	3	7		1	9	
Carcinoid	1	0		0	4	
Nodal involvement						
None	12	36		8	48	
Hilar/mediastinal	10	17	NS, χ^2	5	29	NS, χ^2
pTNM stage						
I	11	32		8	41	
II, III	11	21	NS, χ^2	5	36	NS, χ^2
Smoking history						
Non-smokers	2	4		0	9	
Smokers	20	49	NS, Fisher's test	13	68	NS, Fisher's test
Mean (pack-years)	47.6	42		50.1	41.2	
95% confidence level	14.2	8.5		20.5	6.9	

[a] NS, not significant ($P > 0.05$).

Results

LOH at 5q. Seventy-five of the 108 NSCLC cases studied were informative (heterozygous) for LOH analysis at the *APC* and/or *MCC* loci at 5q21. LOH at the *APC/MCC* gene cluster affected 29% (22 of 75) of these informative NSCLC cases (Fig. 1). It is possible that LOH in one case (case 87) was due to the radiotherapy and chemotherapy given previously as successful treatment for a pulmonary malignant atypical carcinoid tumor. Otherwise, none of the other cases with LOH had received any prior treatment. At individual loci, LOH involved the *APC* locus in 32% (17 of 53) and the *MCC* locus in 22% (9 of 41) of informative cases. Of the five cases informative for both 5q loci, four had concordant LOH at both loci, whereas the other had LOH confined to the *MCC* locus only.

All of the common histological subtypes of NSCLC were affected by LOH at 5q as summarized in Table 1. Similar to a previous study (13), we also found that significantly more SCCs (12 of 32) were affected than adenocarcinomas (4 of 28; $P = 0.04$, Fisher's test). The sex, mean age, and smoking history of cases with and without LOH at 5q were similar (Table 1).

LOH at 5q was significantly associated with biological evidence of tumor progression: (*a*) there was hilar and/or mediastinal nodal involvement in 67% (8 of 12) of SCC cases with LOH at 5q, compared to 25% (5 of 20) of SCC cases without LOH ($P = 0.025$, Fisher's test); and (*b*) NSCLC cases with LOH at 5q had a worse survival than those without LOH ($P < 0.01$, $\chi^2 = 7.85$) (Fig. 2*a*). In addition, this association of LOH at 5q with an adverse outcome was also found in the SCC subtype ($P < 0.02$, $\chi^2 = 5.58$) (Fig. 2*b*). However, we did not demonstrate either a worse outcome or more frequent nodal involvement in adenocarcinomas with LOH at 5q, possibly because of the small number of cases (four) that were affected.

LOH at 18q. LOH was found in 14% (13 of 90) of cases informative at the *DCC* microsatellite marker on 18q (Fig. 1). None of these cases with LOH at 18q had received any prior treatment. In addition, 3 cases demonstrated instability of these microsatellite repeat sequences with either expansion or compression of one allele (data not shown); these were excluded from further clinicopathological and survival analysis. The clinical features including sex, age, and smoking history of cases with and without LOH at 18q were similar (Table 1). In contrast to chromosome 5q, LOH at 18q was not more frequent in SCCs, nor was it associated with nodal involvement or a worse survival (Fig. 2*c*).

Concurrent LOH at 5q and 18q. There were 65 cases of NSCLC which were informative for both chromosomal arms. Of these, 22 were affected by LOH at either or both 5q and 18q. Of these 22 cases, 6 showed LOH on both chromosomal arms, 13 cases had LOH at 5q only, and 3 cases had LOH at 18q only (Table 2).

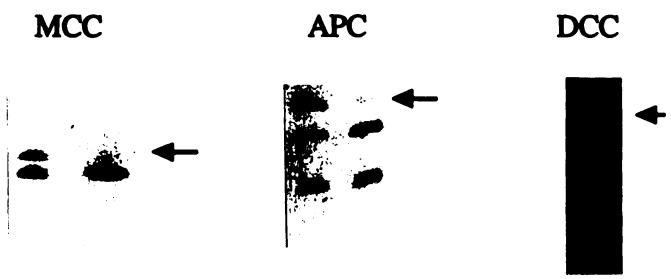

Fig. 1. Representative examples of LOH (*arrows*). In each case, DNA from normal lung and tumor tissue is shown in the left and right lanes, respectively.

Discussion

We report that LOH at chromosome 5q21 occurred in a substantial proportion (29%) of 108 resected NSCLCs. Previously, LOH along 5q was found to affect 21% of a smaller series of 43 resected lung cancers which included 41 NSCLCs (13). Similarly, another study showed *APC/MCC* LOH occurring in 3 of 10 resected NSCLC tumors and 2 of 5 cell lines (12). In contrast, 5q21 deletion was recently reported in 70% of advanced NSCLC cases (3), suggesting that 5q21 is deleted more frequently in advanced cancers and may be important in determining tumor progression in NSCLC. Our data provide strong support for this hypothesis: (*a*) the proportion of resected (*i.e.*, early stage operable cancers) NSCLC with LOH at 5q21 is confirmed at

approximately 30% in a large number of cases comprised of all histological subtypes; and more important, (*b*) the association of LOH at 5q with adverse prognosis as well as nodal progression in SCCs is additional evidence of an important role for 5q loss in tumor progression.

An alternative hypothesis that could account for the higher frequency of LOH at 5q in advanced NSCLC is that the frequent LOH observed merely reflects the random genomic abnormalities of advanced malignancy. However, LOH at 5q in NSCLC is unlikely to be a random phenomenon because it has now been shown to be a consistent finding in a large number of cases from several different studies, and also, despite putative tumor suppressor genes being present on both chromosomes, 5q was far more commonly affected than 18q in the same tumors. Moreover, LOH at 18q in our tumors did not have any of the clinical or prognostic relevance associated with LOH at 5q.

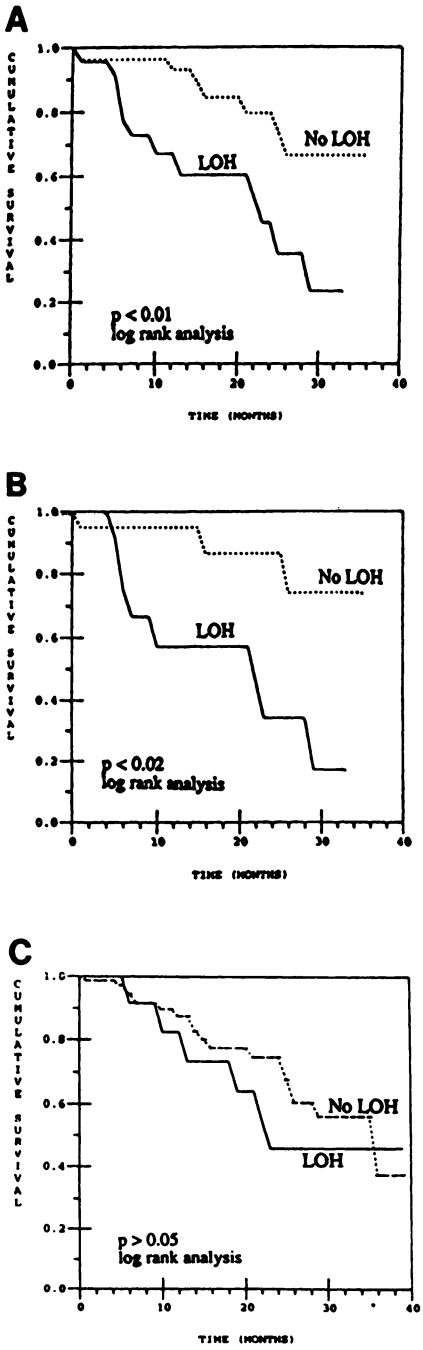

Fig. 2. Actuarial survival curves. *A*, NSCLC cases with and without LOH at 5q21; *B*, SCC cases with and without LOH at 5q21; *C*, NSCLC cases with and without LOH at 18q.

Table 2 *Relationship between LOH at the APC/MCC loci and LOH at the DCC locus in NSCLC cases informative at both chromosomal regions*

	DCC LOH		
	+	−	
APC/MCC LOH			
+	6	13	
−	3	43	$P = 0.015$, Fisher's test

The chromosomal region involved, 5q21, contains the *APC* and the *MCC* genes, both being candidates for the responsible tumor suppressor gene(s) in NSCLC. There is a physiological basis for these loci to be implicated in lung cancer because they are expressed in human and murine lung tissue, respectively (5, 7). Recent data has suggested that the APC protein, when complexed with β-catenin, an adherens junction protein (17, 18), may help maintain intact epithelial layers and thus influence the metastatic process (19).

On the other hand, it is possible that it is actually another tumor suppressor gene at 5q21 apart from either *APC* or *MCC* which is important, and the *APC/MCC* cluster is merely deleted coincidentally. For instance, although the common region of deletion maps to a 3–5-megabase region around the *APC* gene, this region has not yet been finely mapped (3). Moreover, at least two other candidate tumor suppressor regions have been identified on chromosome 5 in lung cancer: one at 5q33–35 telomerically (3) and the other at 5p13–14 (20). Furthermore, mutations (another hallmark feature of tumor suppressor gene inactivation) were not found in the mutation cluster region of *APC* in 55 NSCLC cases, although only 7 of these had allelic loss at 5q (21). Also, it has been suggested that *MCC* does not act as a conventional tumor suppressor in colorectal cancer because *MCC* was rarely deleted independently of *APC*, and the remaining allele was not found to be mutated when one *MCC* allele was deleted (22). Nonetheless, the absence of documented mutations in these genes do not exclude the possibility that loss of one *APC* or *MCC* allele may provide cells with a proliferative advantage through a reduced gene dosage effect.

In marked contrast to 5q, LOH at 18q was far less frequent (14%) and was not associated with histological subtype, TNM stage, or survival in this study. These data imply that LOH at 18q is not of major importance in the pathogenesis or progression of resected NSCLC. However, we did find that LOH at 18q was significantly associated with LOH at 5q in the 65 cases who were informative at both chromosomal arms ($P = 0.015$, Fisher's test; Table 2). Concordance for the presence and the absence of LOH at 5q and 18q was found in 6 and 43 of these cases, respectively. A possible explanation for this association is that both abnormalities may result from a common carcinogen(s), presumably in cigarette smoke. An alternative explanation is that LOH on one chromosomal arm may, for some reason, predispose to LOH on the other chromosomal arm.

The data also provide evidence for genetic heterogeneity within NSCLC subtypes as LOH at 5q affected significantly more SCCs than adenocarcinomas, reminiscent of the frequent activation of the *KRAS* oncogene in adenocarcinomas compared to other subtypes (23). Care should be taken to distinguish between histological subtypes when interpreting genetic studies of NSCLC.

In conclusion, this study has shown that LOH at 5q21 appears to be important in determining tumor progression and may be a useful marker of prognosis in NSCLC, especially in SCCs. The nonradioactive PCR-LOH techniques used to detect LOH at 5q21 may prove to be a rapid and convenient method for its clinical application. Further

study is needed to map and identify the responsible tumor suppressor gene(s) at the 5q21 region in NSCLC.

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