High-Density Marker Analysis of 11p15.5 in Non-Small Cell Lung Carcinomas Reveals Allelic Deletion of One Shared and One Distinct Region When Compared to Breast Carcinomas

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Abstract

The presence of a non-small cell lung carcinoma (NSCLC)-related gene or genes on chromosome band 11p15.5 is of particular interest, given the specific loss of heterozygosity (LOH) measured in this region for many other pediatric and adult neoplasms. We have undertaken high-density polymorphic marker analysis in 30 matched normal and NSCLC tumor samples using 11 PCR-based polymorphic markers positioned approximately every 2-3 cM throughout 11p15.5. These studies have confirmed the presence of two distinct regions of LOH for NSCLC in 11p15.5. In 9 of 13 (69%) tumors with measurable LOH, allelic deletion was restricted to 11p15.5, indicating that whole chromosome 11 loss is not a common event in NSCLC. Furthermore, one-half of these tumors showed independent deletion events for each LOH region, while the remaining tumor regions of LOH extended to include all four markers in between. Only two tumors showed LOH for the more telomeric region alone. Furthermore, the location of these two potentially distinct tumor suppressor genes has been significantly refined to a 3-cM area in the telomeric region between D11S1363 and tyrosine hydroxylase (TH) and a 10-cM area in the more proximal part of 11p15.5 between D11S988 and D11S926. Interestingly, the telomeric region of LOH in NSCLC overlaps with the reported location of one of two breast carcinoma-related tumor suppressor genes, but the proximal allelic deletion area for these two tumor types is clearly distinct. Our studies suggest that chromosome band 11p15.5 harbors a minimum of three separate loci, the loss of which is implicated in these two common adult neoplasms.

Introduction

Lung cancer is now the number one cause of cancer death for both men and women. An estimated 170,000 new lung cancer cases were reported in the United States in 1993; 80% of these are NSCLC (1). The mortality rate of 88% for NSCLC has remained constant since 1985, despite advances in cytotoxic drug development, radiotherapy, and patient management. Presently, there is minimal information about the sequence of events leading to lung cancer, especially for tumors such as adenocarcinomas, which occur in the peripheral airways of the lung. Current theories suggest that as many as 10-20 events, including alterations of oncogenes and tumor suppressor genes, must have occurred by the time lung cancer becomes clinically evident (2). Tumor suppressor genes involved in NSCLC have been located on chromosome bands 3p and 3q (3), 5q (4), 9p (5, 6), 11p (7), 11q (8), and 17p and 17q (9). The presence of such a gene or genes on chromosome 11p and more specifically band 11p15.5 is of particular interest, given the specific LOH measured in this region for many other pediatric and adult neoplasms, including Wilms’ tumors and embryonal rhabdomyosarcomas (10), breast carcinomas (11, 12), and testicular cancers (13, 14). These studies implicate the presence of either a cluster of tumor suppressor genes or a single pleiotropic gene functioning in tumorigenesis in 11p15.5.

In NSCLC, the reported frequencies of LOH on 11p have ranged between 11 and 50% (7, 15-20). The association of 11p LOH with tumor progression and poor patient survival in both NSCLC and breast cancer remains controversial (11, 12, 16, 18). Two different regions of allelic deletion have been identified in 11p15.5 (7), with one area mapping between the markers for HBB and D11S860 and another more telomeric, involving sequences distal to the IGFII locus.

In this current high-density marker analysis, we have measured LOH in 30 matched normal and NSCLC tumor samples using 11 PCR-based polymorphic markers positioned approximately every 2-3 cM throughout 11p15.5. These analyses have confirmed the presence of two distinct regions of LOH in NSCLC and have significantly narrowed the prospective location of these potential tumor suppressor genes to a 3-cM area in the telomeric region between D11S1363 and TH, and a 10-cM area in the more proximal part of 11p15.5 between D11S988 and D11S926. Furthermore, our results indicate that the telomeric region of LOH in NSCLC overlaps with the reported location of one of two breast carcinoma-related tumor suppressor genes (11). However, the proximal allelic deletion area for these two tumor types are distinct (12). This analysis suggests that chromosome band 11p15.5 harbors a minimum of three separate tumor suppressor genes that play a role in these two common adult neoplasms.

Patients and Methods

Patients. Primary NSCLC and patient-matched normal lung tissue samples were obtained from 30 unselected patients upon surgical resection at the UCSD Medical Center, as well as through the Cooperative Human Tissue Network Western Division at Case Western Reserve University. All samples, after surgical removal, were immediately snap-frozen in liquid nitrogen and stored at −80°C until further use. Each fresh lung tumor was histopathologically characterized at the time of surgery, and the corresponding pathology reports have been obtained.

PCR Analysis. DNA from each normal/tumor pair was subjected to PCR analysis using 11 chromosome 11p15.5 markers including D11S2071, D11S1363, D11S922, TH, D11S1318, D11S860, D11S988, D11S1338, D11S909, D11S1346, and D11S926. The likely order of these markers and estimated interval distances are based on both Généthon and Centre d’Etudes du Polymorphisme Humain linkage data (21-23). Detailed amplification information for all polymorphic markers used in this study can be obtained from the Genome Database (Johns Hopkins University Medical School, Baltimore, MD). The conditions for denaturation, annealing, and extension for each primer pair were essentially as reported, except that 0.3-1 unit of Perfect Match (Stratagene) was added to each reaction to optimize the equal amplification of alleles. Primer end-labeling, amplification reactions, and resolution of

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3 The abbreviations used are: NSCLC, non-small cell lung carcinoma; LOH, loss of heterozygosity; TH, tyrosine hydroxylase.
PCR products by denaturing electrophoresis were carried out as described previously (10).

**LOH Analysis.** LOH for lung tumor DNA samples was assessed first by visual inspection, followed by densitometric confirmation as described previously (24). Using a densitometer, the relative ratio of normal and tumor alleles was determined, normalized, and then compared. When the allelic ratio in the tumor DNA was reduced by more than 50% (D0.5) from that found in the normal lung DNA, this sample was noted as having LOH at that locus. Allelic ratios reduced by between 40 and 50% (D0.4-D0.5) were scored as allelic imbalances, and less than 40% (<D0.4) were assigned as maintaining heterozygosity.

**Results**

Matched pairs of normal/tumor lung DNA from 30 NSCLC were assessed for allelic deletions at 11 polymorphic marker loci spanning chromosome band 11p15.5. Our set of tumors included 10 squamous cell, 13 adenocarcinoma, 2 large cell, and 4 unspecified subtypes of NSCLC. Loss of heterozygosity was considered present when the measured allelic ratios in the tumor had decreased by more than 50%. Markers in tumors exhibiting allelic reductions between 40 and 50% where designated as allelic imbalances, and a less than 40% reduction was considered maintenance of heterozygosity. The overall frequency for LOH in 11p15.5 was 45% (13 of 29). Frequencies for individual marker loci ranged from 19 to 52%, with peaks at D11S922 (52%) and D11S1338 (44%) (Fig. 1), declining to an average of 32% for the inter-marker markers. One tumor, 1ST, exhibited microsatellite instability at all markers examined in 11p15.5 except D11S909 and D11S926/data not shown) and was not included in the remaining data analysis. Primary data for the critical tumor samples is shown in Fig. 2, with the corresponding densitometrically determined reductions in allelic ratios. In 8 of 13 (62%) tumors indicating LOH, allelic deletion was restricted to 11p15.5, as indicated by maintenance of heterozygosity at one or more of the most centromeric 11p15.5 markers examined. These results are not evidence of the absence of LOH in more centromeric regions of 11p or 11q because loci in these regions of the chromosome were not analyzed. In fact, several studies in NSCLC have indicated that LOH in other regions of 11p and 11q can be found independently or in conjunction with LOH in 11p15.5 (11, 12, 24). Our findings do indicate however, that whole chromosome 11 loss is not a common event in NSCLC.

Fig. 3 schematically depicts the regions of allelic deletion for the 13 NSCLC tumors in which LOH was identified in 11p15.5. Two distinct regions of LOH in 11p15.5 are revealed. Region 1 (the telomeric region), as shown in tumors 33T and 13T, is defined by markers D11S1363 and TH, an area represented by less than 1 Mb. These results significantly narrow the region containing this proximal tumor suppressor gene. The more proximal LOH Region 2 (less than 2 Mb of sequence) is proximal to D11S988 and distal to D11S926 in tumors 11T and 33T, with the highest LOH frequency observed at marker D11S1338. This positioning represents a 4-cM refinement in location for this second NSCLC-associated gene. Of the eight tumors with allelic deletion confined to 11p15.5, 25% (2 of 8) contained LOH at only the telomeric Region 1, while no tumors (0 of 8) exhibited LOH restricted to the proximal LOH Region 2. In the remaining 6 of 8 (75%) tumors, LOH was measured for both Regions 1 and 2, with one-half (3 of 6) exhibiting LOH in these regions as independent events (e.g., 33T; Fig. 3) and the other one-half exhibiting LOH inclusive of all inter-marker markers (e.g., 84T; Fig. 3). This suggests that a biological preference may exist in NSCLC for altering both regions of 11p15.5 and presumably the corresponding tumor suppressor genes contained within. Alternatively, given the proximity of these two regions, it may also be envisioned that genetic alteration in one region might affect the stability of other nearby sequences via secondary structures generated during mitotic recombination. Such instability could also account for the prevalence of detecting LOH concurrently in two regions of 11p15.5.

In squamous cell carcinoma tumor 100T, a complicated pattern of allelic deletion was discovered. This tumor apparently experienced LOH in three different regions of 11p15.5. The most telomeric region bordered distally by D11S2071 and proximally by TH, overlaps with Region 1 shown in Fig. 3. The allelic ratios were decreased 65-70% in this tumor as compared to normal tissue. The proximal region of LOH in tumor 100T, defined by D11S860 and D11S1338, showed similar allelic reductions. This information alone would suggest that the minimal region containing the proximally positioned tumor suppressor gene for NSCLC might lie in a small 4-cM region between D11S988 and D11S1338. However, an apparent third LOH was measured at marker D11S1338, directly between the other two LOH regions. Densitometric measurements in multiple independent reactions were reproducible, ranging from 37 to 57% and inconsistent with the allelic measurements for the other LOH regions in this tumor. Therefore, 100T was not used to define the minimal regions of LOH found in this set of NSCLCs.

**Discussion**

The results reported here extend and support previous studies indicating that LOH for the short arm of chromosome 11p is a common and important event in human NSCLC. Our high-density marker analysis has identified two distinct regions of LOH within chromosomal band 11p15.5 and has served to significantly refine the location of these putative tumor suppressor genes. Comparison of these results with data from previous studies reveals several important differences. The minimal region of LOH for the telomeric end of 11p15.5 in our tumor samples was defined by a small area bordered by markers D11S1363 and TH. Previous analyses had only been able to place the putative gene in the larger overlapping area between IGFlI and pter (7, 18, 19). Although LOH frequencies for this region were consistent (52%, this report; 45%, Ref. 7), the peak incidence of LOH in this report is highest at D11S922, 6 cM proximal to the peak
Fig. 2. Examples of tumors critical in defining the minimal regions of LOH on chromosome band 11p15.5 in NSCLCs. Autoradiographs represent normal (N) and matched tumor (T) DNA samples from the patients whose case numbers are shown on the left. Chromosome 11p15.5 locus names appear at the top ordered from most telomeric (D11S2071) to most proximal (D11S926). Densitometrically calculated allelic reductions (D) in the intensity of alleles between tumor and normal DNA samples is shown below each autoradiograph. A value greater than 0.5 was indicative of LOH; 0.04–0.05 indicated allelic imbalance; <0.4 was evidence of maintenance of heterozygosity.
Fig. 3. Schematic representation of the regions of allelic deletion measured in 13 NSCLCs. The 11p15.5 loci analyzed are shown telomeric (left) to centromeric (right) along the top. Tumor cases are identified by the numbers shown to the left. Shaded areas indicate the two distinct regions of LOH defined in this analysis. Lines below the figure indicate the minimal region of LOH found in breast carcinomas and their relative position to the NSCLC LOH regions identified in this report. •, loss of heterozygosity; ○, allelic imbalance; □, maintenance of heterozygosity; ○, not informative; ND, not done.

at HRas, reported by Bepler and Garcia-Blanco (7). In the case of LOH Region 2, we found a lower frequency of LOH (44%) as compared to that reported previously (71%). Furthermore, the peak LOH incidence was highest for marker D11S1338, 5 cM distal to the D11S12 locus showing 71% LOH. Curiously, Bepler and Garcia-Blanco (7) found no LOH at either the D11S860 or D11S988 markers, whereas we have measured 30% LOH at each marker in our set of tumors. This discrepancy may reflect the characteristics of the tumor samples analyzed or a difference in interpretation of the corresponding allelic patterns.

The minimal regions of allelic deletion in NSCLC indicate the presence of multiple tumor suppressor genes for adult and pediatric tumors involving sequences in 11p5.5. Many studies support the presence of a gene for embryonal rhabdomyosarcoma and Wilms' tumor (WT2) in 11p15.5 with the shared region of LOH distal to D11S988 (10). Another recent report also localizes a gene involved in malignant gliomas to the area distal to D11S988 (25). This region overlaps with the NSCLC Region 1 reported in this paper. Another study on adenocarcinoma of the stomach identifies a specific region of LOH not overlapping those for NSCLC but corresponding to Region 2 in breast carcinomas (Ref. 26; Fig. 3). Less detailed analyses show general 11p15.5 involvement in a variety of other tumors, including hepatoblastoma and adrenocortical carcinomas (27) and testicular (13, 14), bladder (28), and ovarian carcinomas (29). Furthermore, many inversions and translocations associated with the Beckwith-Wiedemann syndrome and malignant rhabdoid tumors fall within these same affected regions on 11p15.5. Recently established contigs for this chromosomal band (30, 31) propose that the same tumor suppressor genes may be affected by these cytogenetic aberrations in pediatric disorders and neoplasms as well.

In the case of breast carcinomas, recent studies have reported several regions of LOH in 11p15.5 (11, 12). Negrini et al. (11) described two distinct regions of LOH in breast tumors. The telomeric region defined by markers D11S576 and D11S1318 is larger than but encompasses the NSCLC LOH Region 1 shown in this report. It is feasible to predict that the same telomerically located gene is affected in both tumor types. A second LOH region identified in breast tumors, first described by Winqvist et al. (12) is bordered distally by D11S860 and proximally by D11S988. This area is located just distal to the second LOH region described for NSCLC in this report. Both studies by Negrini and Winqvist contain tumors that specifically exclude sequences proximal to marker D11S988 as involved in breast cancer. This is the first evidence indicating that two independent tumor suppressor genes exist in the proximal region of 11p15.5 which may function in a tissue-specific manner in the tumorigenic process. Only the detail achieved by this high-resolution allelic deletion analysis has been successful in defining these three distinct tumor suppressor regions.

Our data show retention of heterozygosity for at least one marker on 11p in 12 of 13 (92%) of tumors exhibiting LOH, indicating that loss of the whole chromosome 11 is a rare event in NSCLC. This is in contrast to the events leading to LOH on chromosome 3 where...
whole chromosome loss, rather than deletion, appears to be the underlying mechanism in at least one of three patients with NSCLC and allelic loss on 3p (32). The presence of multiple LOH regions in 11p15.5 for both lung and breast tumors presents a scenario where LOH at these different loci could potentially occur on the same chromosome 11 concurrently or on separate chromosomes. Previous studies have demonstrated a paternal origin for the remaining alleles in rhabdomyosarcomas (33) and Wilms’ tumors (34), suggesting that LOH occurs on the same chromosome 11p. However, these events represent singular LOH lesions, unlike the two distinct regions found in most of the NSCLCs analyzed here. To date, only one Wilms’ tumor has exhibited two independent regions of LOH (10), and these occurred in separate bands (11p15.5 and 11p13). In breast carcinomas, tumors have been found that exhibit 11p15 and 11q LOH at varying frequencies, indicating that tumors can in fact be heterogeneous for these events and suggesting that multiple genetic events affecting the same chromosome can act independently of one another, contributing to the stepwise development of the fully malignant phenotype. It would be of particular interest to analyze lung tumors with distinct regions of LOH with regard to their inherited haplotype to determine if the distinct LOH regions are in fact independent events occurring on the same or separate chromosome 11s.

The clinical relevance of LOH on 11p15.5 remains unclear. A review of the literature on lung cancer has shown no significant difference in the frequency of allelic deletion between the adenocarcinoma and squamous cell carcinoma subtypes. The overall average frequency of 11p15 LOH was determined to be 33% (27 of 82) for adenocarcinomas, 36% (39 of 108) in squamous cell carcinomas, and 33% (4 of 12) for large cell carcinomas (this report; Refs. 7 and 15–20). Attempts at clinical correlations also remain controversial. Fong et al. (16) examined 101 cases of NSCLC and found LOH along lip (not specifically 11p15.5) to be correlated with poor survival after metastasis. Cancer Res., 55: 2660–2664, 1995.

References
ALLELIC DELETION OF 11P15.5 IN LUNG CANCER


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