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Advances in Brief

Microsatellite Instability and Other Molecular Abnormalities in Non-Small Cell Lung Cancer[1]

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Abstract

Microsatellites are highly polymorphic, short-tandem repeat sequences dispersed throughout the genome. Instability of these repeat sequences at multiple genetic loci may result from mismatch repair errors and occur in hereditary nonpolyposis colorectal carcinoma and certain sporadic cancers. In non-small cell lung cancer, we found that microsatellite instability was infrequent, affecting only 7 (6.5%) of 108 cases. Despite being observed in all histological subtypes and at different tumor stages, microsatellite instability most commonly affected only one of the six loci tested on five chromosomal arms. In addition, microsatellite instability was associated with extensive, concurrent molecular changes including K-*ras* and *p53* mutations as well as frequent loss of heterozygosity at chromosomal regions 5q, 8p, 9p, 11p, and 17p.

Introduction

Microsatellite instability, representing mutations of the short-tandem repeat sequences distributed within the genome, were initially reported in sporadic colorectal cancers and HNPCC[3] (1–3). Microsatellite instability appears to be a novel molecular mechanism in carcinogenesis and is thought to reflect multiple replication errors from abnormalities of the mismatch repair genes, including *hMSH2* and *hMLH1* (4–10). Besides colon cancer, microsatellite instability has also been described in a variety of other tumors, both sporadic and familial cancers (11–18).

In SCLC, 45% of primary cancers were found to have microsatellite instability in the form of deletion or expansion of dinucleotide repeats (19). However, in the other major subtype of lung cancer, NSCLC, the data on microsatellite instability has been conflicting in terms of the frequency and pattern of microsatellite instability. One study showed that unlike cancers of the HNPCC spectrum where instability involves multiple genetic loci, only one single microsatellite locus was altered in 2 (2%) of 87 NSCLC cases (20). Similar data were reported in a study of about 400 non-HNPCC syndrome tumors including an unspecified number of NSCLCs, in which between 1 and 3% showed instability at a single dinucleotide marker (19). Significantly different results were reported however in another study of 38 NSCLC where instability occurred in a much higher percentage (34%) and affected several microsatellite markers concurrently (21).

We studied 108 cases of resected NSCLC for microsatellite instability at six genetic loci on five chromosomes using a variety of microsatellite types including four dinucleotide, one tetranucleotide, and one AluVpa (22) marker. Instability, which was found in seven cases, usually affected only one locus and was associated with a high frequency of other molecular genetic abnormalities.

Materials and Methods

Samples (n = 108) of resected tumors and corresponding normal lung tissue were obtained from surgically treated patients with NSCLC. There were 46 cases of adenocarcinomas, 43 squamous cell carcinomas, 11 mixed adenosquamous tumors, 4 large cell tumors, and 4 carcinoids. Independent histological examination of the tumors was performed according to 1982 WHO criteria. All patients underwent detailed postoperative pathological TNM staging.

DNA was extracted from snap-frozen tumor and normal lung tissue using standard techniques. Tumor and normal DNA were amplified by PCR at six microsatellite markers: chromosome 2p, *D2S123* (dinucleotide); chromosome 9p, *D9S126, IFNA* (dinucleotides); chromosome 18q, *DCC* (dinucleotide); chromosome 8p, *LPL* (tetranucleotide); and chromosome 1p, *MYCL* (AluVpA). PCR mixtures were 20 μl and incorporated 2.4 μCi [^{35}S]dCTP. PCR conditions were initial denaturation at 94°C for 3 min, 30 cycles of 92°C for 45 s, 55°C (46°C for *DCC*) for 90 s, and 72°C for 90 s. Final extension was 72°C for 7 min. PCR products were denatured by 95% formamide and electrophoresed on 7 M urea polyacrylamide gels for 2–3 h at 40 W followed by autoradiography. Negative controls were incorporated with each PCR run. Tumor microsatellite instability appeared as additional bands of differing sizes compared to normal DNA, and LOH affecting informative samples was scored by two independent observers. Abnormal samples were repeatedly tested in independent PCR reactions and separate gel loadings to ensure reproducibility; identical alleles were noted on each occasion.

To detect K-*ras* mutations at codon 12, a PCR technique based on two rounds of amplification for mutant enrichment and *Bst*N1 digestion to distinguish between normal and mutant alleles was used (23). Mutated codon 12 sequences were confirmed and characterized by direct dye-primer automated sequencing in each case (Applied Biosystems 370A DNA sequencer).

Mutations of *p53* exons 5–8 were detected by SSCP analysis (24), and paired normal and tumor samples were repeatedly tested to ensure reproducibility. Briefly, the primers used were: exon 5 (TTCCTCTTCCTGCAGTACTC and GCAAATTTCCTTCCACTCGG), exon 6 (GCCTCTGATTCCTCACTGAT and TTAACCCCTCCTCCCAGAGA), exon 7 (ACTGGCCTCATCTTGGGCCT and TGTGCAGGGTGGCAAGTGGC), and exon 8 (TAAATGGGACAGGTAGGACC and TCCACCGCTTCTTGTCCTGC) end labeled with [^{32}P]dATP using T4 polynucleotide kinase (25, 26). Products were denatured at 94°C, and electrophoresed on a 5% polyacrylamide gel for 2–3 h at 40 W followed by autoradiography.

LOH was examined at the *D17S5* locus on chromosome 17p13 by RFLP/Southern blot analysis with the pYNZ22 probe (27), at the H-*ras, INS, RRM1, FSHB, WT1,* and *CAT* loci on 11p13–11p15 by RFLP analysis as described previously (28), and at the *MCC* and *APC* loci on 5q using PCR analysis based on an insertional and *Rsa*I polymorphism, respectively (29, 30).

Results

Microsatellite Instability in Lung Tumors. Of 108 cases of resected NSCLCs, 7 demonstrated microsatellite instability. The instability appeared as either expansion or compression of a single band or a "ladder" of bands (Fig. 1). Only three of the six microsatellite loci examined, *D2S123* (dinucleotide, two cases), *DCC* (dinucleotide,

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[3] The abbreviations used are: HNPCC, hereditary nonpolyposis colorectal cancer; NSLC, non-small cell lung cancer; SCLC, small cell lung cancer; SSCP, single-stranded conformational polymorphism; LOH, loss of heterozygosity.

Table 1 *Microsatellite instability in lung tumors*

Case	Subtype[b]	Age (years)	TNM stage	Microsatellite instability[a]						K-*ras* codon 12 mutation	*p53* SSCP mutation	*D17S5*	11p13–11p15	*MCC/APC*
				IFNA	*D9S126*	*DCC*	*D2S123*	*LPL*	*MYCL*					
8	AS[b]	64	II	L	ho	—[c]	ho	ho	L		exon 6	L	L	ho/he
14	AC	71	III	L	ho	—	ho	L	—			L	he	ho/ho
30	SCC	72	I	ho	ho	he	—	L	ho	GTT	exon 8	ho	he	ho/he
33	SCC	70	III	L	L	he	L	L	—		exon 5	L	L	ho/L
57	SCC	47	III	he	he	L	—	he	L			L	he	ho/ho
75	BAC	54	I	L	ho	—	he	he	he	GAT		L	L	ho/L
100	AS	71	I	ho	L	he	he	L	—			L	L	he/ho

[a] Cases with microsatellite instability. Genetic loci or chromosomal regions are listed in the top row.
[b] AS, adenosquamous carcinoma; SCC, squamous cell carcinoma; AC, adenocarcinoma; BAC, bronchioloalveolar carcinoma; L, loss of heterozygosity; he, heterozygous; ho, homozygous.
[c] —, positive for microsatellite instability.

three cases), and *MYCL* (AluVpa, three cases), demonstrated instability. Although instability may occur more commonly in trinucleotide and tetranucleotide rather than dinucleotide repeats (11), we did not detect it at the *LPL* tetranucleotide in this study. In six of the seven cases, microsatellite instability affected only one of the six microsatellite loci tested. In case 14, however, instability involved two separate loci concurrently, *DCC* and *MYCL* (Table 1).

Microsatellite instability was found (*a*) in all histological subtypes, squamous cell carcinoma (three cases), adenosquamous carcinomas (two cases), and adenocarcinoma and bronchioloalveolar carcinoma (one case each), and (*b*) at all tumor stages, Stage I (three cases), Stage II (one case), and Stage III (three cases). Similar ages and smoking histories were noted in both the groups with and without instability (Table 2). Although all of the tumors with instability were in males, this sex difference was not statistically significant when compared to the cancers without instability in the remaining 69 males and 32 females ($P = 0.102$, Fisher's test).

Correlation with K-*ras* and *p53* Mutations. The seven tumors with microsatellite instability were examined for mutations in codon 12 of the K-*ras* gene using a PCR/restriction enzyme method and direct sequencing, and in the *p53* gene using SSCP analysis of exons 5–8. Two cases had K-*ras* codon 12 mutations, changing the normal GGT sequence to GTT and GAT, respectively. Three cases had mutations in *p53* exons 5, 6, and 8, respectively, as evidenced by altered tumor band mobility on the SSCP gels in comparison to corresponding normal DNA.

Correlation with LOH at 5q, 8p, 9p, 11p, and 17p. All affected tumors were examined for LOH at chromosome 5q (*MCC/APC* loci), 11p (H-*ras*, *INS*, *RRM1*, *FSHB*, *WT1*, and *CAT*), and 17p (*D17S5*). In addition, the microsatellite markers were also used to determine LOH on chromosomes 8p (*LPL*) and 9p (*IFNA* an d*D9S126*). LOH was found in two of five informative cases at the *MCC/APC* gene cluster, four of six informative cases at chromosome 8p, five of six informative cases at 9p, four of seven informative cases at 110, and all six informative cases at 17p (Table 1).

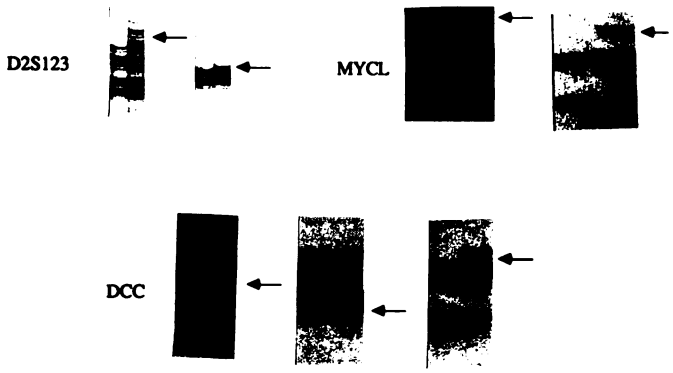

Fig. 1. Examples of microsatellite instability at the *D2S123*, *DCC*, and *MYCL* loci. *Arrows* refer to novel bands representing altered (expanded or deleted alleles). In each case, the *left lane* contains normal DNA and tumor DNA is in the *right lane*.

Table 2 *Clinical features of cases with microsatellite instability*

	Cases with instability	Cases without instability
Sex		
Male	7	69
Female	0	32
Age		
Mean (yr)	64.1	60.8
95% confidence interval	7.3	2.1
Smoking history		
Mean (packs-yrs)	44.3	41.9
95% confidence interval	23.2	6.1

Discussion

In this study of resected NSCLCs, microsatellite instability only affected 6.5% of 108 cases. Our results support a previous study which suggested that microsatellite instability in NSCLC was infrequent (2%) and restricted to one marker at a time (20). However, these data differ from another study of 38 NSCLCs where microsatellite instability was not only frequent (34%) but also often affected multiple markers concurrently (21).

One explanation proposed for the different frequencies of microsatellite instability reported in the two earlier studies (2% *versus* 34%) was that microsatellite loci on various chromosomes may have differing frequencies of instability (20, 21). For instance, only 1 of 8 loci in the former study were situated on chromosome 3 compared to 11 of 16 loci in the latter study. Thus, if chromosome 3 microsatellites were preferentially affected by instability, this could account for the much higher frequency of instability in the latter study. Evidence against this hypothesis, however, is the finding that microsatellite instability often affected other chromosomes apart from chromosome 3 in SCLC (19). Furthermore, our study only tested microsatellites which were known to be capable of demonstrating instability, *i.e.*, *IFNA* and *D9S126* in SCLC (19) and the others in colorectal cancers[4] (31). Thus, we believe that our finding of a low frequency of instability in NSCLC is valid and not just due to differences of the intrinsic susceptibility of various microsatellite markers to instability.

A second point is that the pattern of instability showed that in most of our cases affected, just one microsatellite locus was affected. Again, this is similar to one study (20) but not the other where instability affected single and multiple loci concurrently (21). The reasons for these differences are unclear. Perhaps there are geographical and/or ethnic factors which cause the multiple loci involvement seen in the North American

[4] J. Young, personal communication.

cases (21) that are not found in the Norwegian (2) or Australian cases, which tend to have single locus involvement. Geographical differences are known to exist for various chromosomal aberrations in tumors (32). Another possible explanation is that microsatellite instability is associated with advanced tumor stage and cancers of different stages were analyzed in the contrasting studies. However, this seems unlikely as two studies (21 and current) have shown that microsatellite instability was present in a range of TNM stages.

In addition, there is evidence that the pattern of microsatellite instability also varies between different tumor types, a factor which adds to the complexity of this abnormality. In many cancers, including colon (1–3, 12–14, 17) and endometrial (15) tumors, microsatellite instability affects multiple genetic loci concurrently. On the other hand, instability may only affect a single locus in certain cancers, such as breast and liver (16) as well as sarcoma, brain, and ovarian tumors (11). Many of the cancers with microsatellite instability at multiple loci tend to be associated with HNPCC (33). Conversely, the cancers with single locus instability, such as lung cancer, tend not to be associated with the HNPCC syndrome.

We also found a very high frequency of LOH affecting other chromosomes, particularly 11p (57%), 8p (66%), 9p (83%), and 17p (100%) in all 7 tumors with microsatellite instability, frequencies which are higher than expected in NSCLC. Although the study with contrasting results discussed earlier (21) showed that only 29% of NSCLCs with instability had LOH at 3p, a similar association of frequent allelic loss with microsatellite instability was also found in SCLCs (19). In marked contrast however, microsatellite instability correlated inversely with allelic loss in sporadic colon cancers (1, 2). These data taken together strongly suggest that microsatellite instability has different roles in lung and colon cancer.

In addition to LOH, we found that the cases with microsatellite instability also had frequent mutations affecting K-*ras* codon 12 (2/7) and the *p53* gene (3/7). In fact, these mutation frequencies are probably conservative as a small proportion of mutations occur at other K-*ras* codons and outside of *p53* exons 5–8, respectively.

Our data show that microsatellite instability in NSCLC was associated with widespread genomic abnormalities. One explanation for this and the infrequency of instability may be that instability in NSCLC may merely reflect extensive genetic damage, rather than play a major pathogenic role. However, an alternative hypothesis which requires testing is that in a small subgroup of NSCLCs, microsatellite instability may in some way predispose to further genetic damage.

In any case, these findings suggest an apparent difference in the basic mechanisms of carcinogenesis between familial HNPCC-associated cancers and sporadic cancers. In many of the tumor types associated with HNPCC, microsatellite instability appears to play an important role whereas in NSCLC, a sporadic cancer caused mainly by cigarette smoke, with little familial tendency, instability appears far less important. It would be important to investigate why results differ in the reported NSCLC studies and why microsatellite instability appears to be far more common in the other major subtype of lung cancer, SCLC.

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