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Allele Diversity of the H-*ras*-1 Variable Number of Tandem Repeats in Norwegian Lung Cancer Patients

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We have examined restriction fragment length polymorphisms of the H-*ras*-1 gene in germ-line DNA from 214 lung cancer patients and 309 unaffected controls. When DNA samples were digested with *Msp*I/*Hpa*II, Southern blot analysis revealed at least 22 different alleles, grouped according to their frequencies as common, intermediate, and rare. The frequency of rare alleles in lung cancer patients (16/428) is significantly different ($p = 0.002$) from that in the control group (5/618). Individuals with rare alleles were found to be at 4.7-fold greater risk of lung cancer than those with no rare alleles.

Introduction

The H-*ras*1 gene is associated with restriction fragment length polymorphism (RFLP) resulting from a variable number of tandem repeats (VNTR) of a 28-bp consensus sequence in the 3′-flanking region. Several studies have focused on this allelic variation in the search for genetic markers of cancer susceptibility. RFLP studies by Krontiris et al. (*1*) demonstrated a significantly higher frequency of rare H-*ras* alleles in a mixed group of cancer patients than among the controls. Several investigators have failed to reproduce this observation for specific cancer types (*2,3*). Statistical analysis of some of these studies has supported the suggestion that the reported association between rare H-*ras* alleles and cancer susceptibility may have occurred by chance (*4*). Furthermore, the hypothesized relationship does not yet have a mechanistic basis. In a previous RFLP study of the H-*ras* gene in germ-line DNA from 118 lung cancer patients and 123 unaffected controls, we reported a significantly higher frequency of rare alleles in the lung cancer group (*2*). These results are in agreement with the finding of Sugimura et al. (*3*) among U.S. lung cancer patients. In the present study, we have extended the groups to comprise 214 cancer patients and 309 controls.

Materials and Methods

The lung cancer group comprised 214 patients of Norwegian origin with untreated primary lung carcinomas. The control group consisted of 309 healthy individuals living in eastern and central Norway, all of Norwegian origin. The groups were not matched with respect to smoking habit or age.

DNA extraction and Southern blot analysis were performed essentially as described previously (*2*). The chi-square test with Yates' correction was used for the statistical analysis.

Results

We investigated the *Msp*I/*Hpa*II polymorphism of the VNTR region flanking the H-*ras*1 locus (Table 1) and found at least 22 different alleles. They were grouped as common, intermediate, and rare according to their frequencies in the control group and criteria used by Krontiris et al. (*1*). The incidence of rare alleles in the lung cancer group (16/428) is significantly greater ($p = 0.002$) than that in controls (5/618) (odds ratio = 4.76; 95% confidence interval, 1.65–16.7). Disregarding the grouping, there is a pronounced scattering of alleles on many different fragment lengths in the lung cancer group. The small variation in the fragment lengths of alleles typed as 2.08-kb and 2.57-kb also seemed to be more frequent in the lung cancer group than in controls; however, this small varia-

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Table 1. Comparison of H-*ras*-1 alleles in lung cancer patients and in unaffected controls.

Allele, kb	Lung cancer cases		Controls		
	No.	%	No.	%	*p*-Value
1.00	257	60.0	416	67.3	
1.45	47	11.0	63	10.2	
2.08[a]	61	14.3	58	9.4	
2.57[a]	20	4.7	52	8.4	
Total common	385	90.0	589	95.3	0.01 (df=3)
0.97	5	1.2	5	0.8	
1.03	6	1.4	5	0.8	
1.06	4	0.9	6	1.0	
1.11	10	2.3	4	0.6	
1.42	2	0.5	4	0.6	
Total intermediate	27	6.3	24	3.9	NS
1.09	–		1	0.2	
1.17	2	0.5	–		
1.36	1	0.2	1	0.2	
1.48	2	0.5	–		
1.50	3	0.7	1	0.2	
1.57	1	0.2	–		
1.71	1	0.2	–		
1.80	1	0.2	–		
1.83	1	0.2	–		
2.37	–		1	0.2	
2.51	2	0.2	1	0.2	
2.96	1	0.2	–		
3.05	1	0.2	–		
Total rare	16	3.7	5	0.8	0.002 (df=1)

Abbreviations: NS, not significant; df, degrees of freedom.
[a]Includes alleles that may deviate by about 1 tandem repeat unit (28 bp) from the indicated fragment length.

tion of about one tandem repeat unit (28 bp) is close to the resolution limit of our system. The exact size of these allele types is uncertain. Using a second restriction enzyme endonuclease (*Ava*II), which detects the same polymorphism of the *ras*-VNTR region as *Msp*I/*Hpa*II, we have excluded the existence of rare or intermediate alleles resulting from restriction site polymorphisms.

Discussion

In this RFLP study of the VNTR region flanking the H-*ras*1 gene, we have demonstrated significantly increased incidence of rare alleles among lung cancer patients compared to unaffected controls. Comprising 214 patients and 309 controls, this study is so far the largest performed on lung cancer. Our results confirm those of a similar study by Sugimura et al. (*3*) of white and black lung cancer patients in the United States. The frequency of rare alleles in Norwegian and U.S. populations seems to be different, in both the lung cancer and control groups; however, the relative risk for lung cancer associated with rare alleles is similar (~4).

Generally, the allelic variability of VNTR loci may differ widely from one locus to another. These differences are probably associated with different mutation rates to new alleles of different lengths *(5)*. The pronounced diversity of H-*ras* VNTR alleles in the lung cancer group that we studied may thus reflect increased instability of this locus in this subpopulation. Our hypothesis is that a susceptibility gene(s) is involved in the instability of the *ras* VNTR region. This predicts that the susceptibility gene(s) may segregate differently from the mutated VNTR allele. Thus, only the newly originated (mutated) VNTR alleles may be reliable markers for susceptibility, whether they are classified as rare, intermediate, or common alleles in the population, although these new mutations are statistically most often found in the group of rare alleles.

With regard to the mechanisms for generating new VNTR alleles at the H-*ras* locus, unequal cross-over during meiotic recombination seems to be excluded *(6)*. There is some evidence that the rare alleles are most often generated from the common allele of nearest fragment length *(6)*. Thus, the majority of rare alleles have fragment lengths scattered near those of the most common alleles.

A number of case-control studies have failed to demonstrate any association between rare H-*ras* alleles and specific types of cancer *(2,3)*. Several of these studies were not performed optimally, however, since sources of rare alleles other than genetic instability were not considered; these might include ethnic heterogeneity in the case-control groups or restriction site polymorphisms within the VNTR region. It may well be, however, that the H-*ras* protooncogene or the susceptibility gene(s) is not essential in the development of some of these cancers. In our newly performed investigation of H-*ras* VNTR alleles in testicular cancer patients, we found a high and significantly increased incidence of rare alleles (Rybert et al., manuscript in preparation). Rare VNTR alleles at the H-*ras* locus are therefore not unique markers for lung cancer susceptibility.

It is commonly accepted that cigarette smoking is the leading risk factor for lung cancer. The hypothesis that increased cancer susceptibility is related to the presence of rare *ras* alleles predicts that the average smoking history of lung cancer patients with rare alleles may be different from that of patients without rare alleles. Unpublished data from our laboratory confirm this prediction.

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