Microsatellite instability in patients with chronic obstructive pulmonary disease

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Abstract. Chronic obstructive pulmonary disease (COPD) is a relatively common disease, affecting mainly males in the western world. Although substantial data are available as regards the clinicopathological characterization of COPD, little is known of the molecular basis of the disease. In the present study we analysed the incidence of microsatellite instability (MI) in cytological specimens from patients with COPD. MI reflects increased mutational rate and is associated with decreased accuracy in the DNA repair, resulting in the accumulation of somatic mutations in cells manifesting this genetic alteration. Among 31 specimens tested, 7 (23%) exhibited MI in at least one among 6 microsatellite markers tested. 5 cases were affected in only one marker while the remaining two cases exhibited evidence of MI in two microsatellite markers. These data suggest that an elevated mutational rate as reflected by the increased incidence of MI is associated with the development of the disease.

Introduction

Chronic obstructive pulmonary disease (COPD) is a disorder characterised by reduced maximum expiratory flow and slow forced emptying of the lungs: features which do not change markedly over several months. Most of the airflow limitation is due to varying combinations of airway disease and emphysema. It is difficult to define *in vivo* the relative contribution of the two processes (1,2).

The two major diseases classified as COPD are emphysema and chronic bronchitis. Both are histologically characterised by increased wall thickening, increased intraluminal mucus and changes in the lining fluids of the small airways. Emphysema is defined anatomically by permanent destructive enlargement of airspaces distal to the terminal bronchioles without obvious fibrosis (3). In contrast chronic bronchitis is defined by the presence of chronic or recurrent increases in bronchial secretions sufficient to cause expectoration. This hypersecretion can occur in the absence of airflow limitation (4,5).

It is of interest to note that most of the patients with COPD are or were cigarette smokers. The two main symptoms that cause patients with COPD to consult a physician are breathlessness and cough, sometimes accompanied by wheezing or sputum production. A history of repeated respiratory infections, especially during the winter, is common. Most patients are long-term cigarette smokers.

Although several clinical studies exist on the pathophysiology of COPD, little is known as regards the molecular basis of the disease. In the present study we performed an assessment of the mutational rate in patients with COPD, as reflected by the incidence of microsatellite instability (6).

Instability of tandem repeat DNA sequences or MI, is a genetic disorder that occurs in many tumours (7-14) and it has been correlated with a high mutational rate (15). Mutations in genes involved in DNA repair has been aetiologically associated with this disorder (16).

In the present study we distinguished the molecular basis of the COPD disease. We investigated the incidence of MI in 31 patients with COPD and whether this particular alteration is detectable in cytological specimens. We found that 7 (23%) cases exhibited MI suggesting that elevated mutational rate is associated with the development of the disease.

Materials and methods

Specimens and DNA extraction. Specimens were obtained from the University Hospital, Department of Pulmonary Medicine, Heraklion, Greece. DNA from the peripheral blood was extracted by the Nucleon kit (Scotlab) following the manufacturer's instructions. DNA from cytological material was extracted by the standard boiling method and ethanol precipitation. DNA samples were stored at 4°C.

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Table I. Incidence of microsatellite instability and chromosomal location for the 6 microsatellite markers used.

Marker	Chromosome	Cases with alteration (%)
D3S1234	3	0
D3S1210	3	2 (6)
D6S344	6	1 (3)
HRM	11	2 (6)
THRA1	17	3 (10)
D17S250	17	1 (3)

Table II. Microsatellite instability in patients with COPD.

Patient no.	D3S1234	THRA1	D6S344	D17S250	HRM	D3S1210
1	-	-	-	-	+	-
2	-	-	-	-	-	-
3	-	-	-	-	-	+
4	-	-	-	-	-	-
5	-	+	-	-	-	-
6	-	-	-	-	-	-
7	-	-	-	-	-	-
8	-	+	-	-	-	-
9	-	-	-	-	-	-
10	-	-	-	-	-	-
11	-	-	-	-	-	-
12	-	-	-	-	-	-
13	-	-	-	-	-	-
14	-	-	-	-	-	-
15	-	-	-	+	-	+
16	-	-	-	-	+	-
17	-	-	-	-	-	-
18	-	-	-	-	-	-
19	-	-	-	-	-	-
20	-	-	-	-	-	-
21	-	-	-	-		-
22	-	-	-	-	-	-
23	-	-	-	-	-	-
24	-	-	-	-	-	-
25	-	-	-	-	-	-
26	-	-	-	-	-	-
27	-	-	-	-	-	-
28	-	+	+	-	-	-
29	-	-	-	-	-	-
30	-	-	-	-	-	-
31	-	-	-	-	-	-

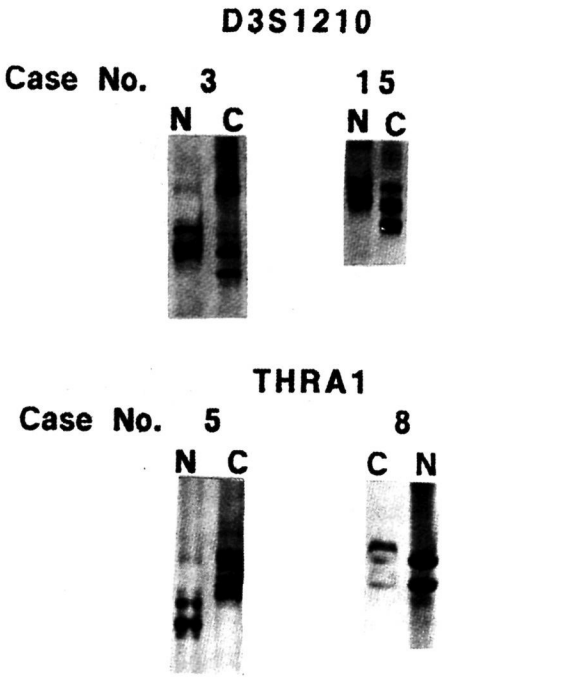

Figure 1. Representative examples of specimens exhibiting microsatellite instability. N and C correspond to peripheral blood and cytological specimens respectively.

Microsatellite analysis. Six microsatellite markers located on 4 different chromosomes were used. PCR reactions were performed in a 12.5 µl reaction volume containing approx. 100 ng of genomic DNA, 500 µM dNTPs, 10 pmol of each forward and reverse primer, 1.25 µl of 10X buffer [670 mM Tris-HCl pH 8.5, 166 mM ammonium sulphate, 67 mM $MgCl_2$, 1.7 mg/ml bovine serum albumin, 100 mM ß-mercaptoethanol and 1% (w/v) Triton-X-100] and 0.3 U *Taq* polymerase. The reactions were denatured for 5 min at 95°C and the DNA was subsequently amplified for 28 cycles at 95°C, 58-60°C and 72°C each step. 5 µl of the PCR product was electrophoresed in a 10% polyacrylamide gel and silver stained. MI was scored by comparing the electrophoretic pattern of the microsatellite markers amplified from the paired DNA preparations that corresponded to the cytological specimens and the peripheral blood sample. The analysis in the MI positive cases was repeated at least twice and the results were highly reproducible.

Results and Discussion

Thirty-one cytological specimens from patients with COPD were analysed for MI using a bank of 6 polymorphic microsatellite markers (Table I). The electrophoretic pattern of each specimen was compared with the corresponding pattern of the peripheral blood and any difference in the mobility of the microsatellite alleles was interpreted as MI. Among 31 cases tested, 7 (23%) were interpreted as MI-positive. In 5 of these cases instability affected only 1 marker while in the remaining 2 cases, 2 markers were affected (Table II, Fig. 1).

A methodological limitation of the current analysis is that cytological samples have a considerable quantity of normal DNA. This results in a competition between the novel microsatellite alleles produced by the instability and the normal alleles, which might eliminate the signal of the mutant alleles. This may decrease the figures of the present study since false negative results may be obtained. However, 23% of the specimens exhibited MI suggesting that this

particular alteration is a detectable phenomenon in the development of the disease.

Microsatellite instability initially was detected in hereditary non polyposis colorectal cancer and later extended to almost all human sporadic tumours including lung, endometrial, colorectal, head and neck and prostatic tumours (7-14). In addition, recent studies suggested that MI is also a detectable phenomenon in spontaneously aborted embryonic tissues (17-19). MI is the direct result of decreased accuracy in DNA repair and replication and it reflects an elevated mutational rate which indicates destabilization in the genome. The detection of MI in patients with COPD suggests that the disease proceeds through mutations which consequently activate or inactivate other genes involved in the pathogenesis of the disease. However, the potential targets of these mutations remain obscure since the molecular basis of the disease is unclear as yet.

An additional point of the present study is that it demonstrates that the detection of MI is possible in cytological material. This may find clinical application in patients with lung cancer (20,21) since the disease is characterised by a high incidence of MI and alterations in oncogenes and onco-suppressor genes (22).

The present study suggest that COPD is characterised by a considerable incidence of MI and that this particular genomic alteration is detectable in cytological specimens. Future studies are required in order to reveal the precise significance of these findings and their potential role in the clinical practice.

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