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Microsatellite Instability in Human Atherosclerotic Plaques

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The aetiopathology of atherosclerosis remains obscure. Although histologically the accumulation of lipids and the proliferation of the smooth muscle cells represents the main feature of the disease, little is known as regards the molecular alterations associated with the atherosclerotic lesions. In the present study we investigated whether an elevated mutational rate is detectable in human atheromatous plaques. Thirty specimens were assessed for microsatellite instability (MI) by 7 microsatellite markers and MI, in at least one marker, was apparent in 6 (20%) cases. Our data suggest that decreased fidelity in DNA replication and repair may be associated with the development of the disease.

The accumulation of lipids and the proliferation of smooth muscle cells are considered as the main histological feature of the atherosclerotic plaques (1). At the sub-cellular level recent studies have recognised particular molecular alterations to play an important role in the development of the disease, such as aberrant expression of growth factors and cytokines (1,2) as well as the presence of viruses in the atheromatous plaque DNA (3). In addition, atherosclerotic plaques have a monoclonal origin (4) and the presence of transforming oncogenes has been reported (5,6) although other groups failed to confirm these data (7). The forementioned observations led to the suggestion that the atherosclerotic plaques possess similarities with neoplasia and thus they should be considered as a neoplastic benign lesion (8).

A recently discovered feature of the neoplastic cells is the elevated mutational rate which is reflected in the instability of the microsatellite DNA (microsatellite instability, MI) (9). MI was initially reported in colorectal cancer (HNPCC) (10–12) and later extended to almost all human tumours (13–16), neurodegenerative diseases (17,18) as well as spontaneously aborted embryonic tissues (19–21).

The aim of our study was to analyse the incidence of MI in atherosclerotic tissues. If indeed the molecular basis of the disease is similar to the development of neoplasia, then MI should be detectable in these lesions and might also be associated with the presence of transforming oncogenes in particular cases. Our data show that MI occurs in atheromatous plaques and that an elevated mutational rate may be associated with the development of the disease.

MATERIALS AND METHODS

Specimens and DNA extraction. Specimens (17 males and 13 females) were obtained from autopsy cases, ranging from 60 to 79 years in age, from the Laboratory of the Public Forensic Pathology Service, Athens. All patients died of myocardial infraction. The plaques were selected to be not calsified and measured around 0.5 cm in diameter. Histologically all specimens contained foam cells as the main component. Calsified specimens and the specimens with significant fibrous components were excluded from the study. Twenty specimens were taken from the aorta and ten specimens from the basilar cerebral artery. The tissue specimens were frozen in liquid nitrogen immediately after excision and stored until DNA extraction.

Genomic DNA was extracted from the frozen tissues as previously described (13). DNA samples were stored at 4°C.

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Microsatellite analysis. Seven microsatellite markers each located on a different chromosome (13,22) were used (Table 1). 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 β-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 atherosclerotic plaque with adjacent normal tissue. The analysis in the MI positive cases was repeated at least twice and the results were highly reproducible.

RESULTS AND DISCUSSION

The incidence of MI was analysed in 30 atheromatous tissues using a bank of 7 polymorphic microsatellite markers. MI was scored by comparing the electrophoretic pattern of the microsatellite sequences in the atheromatous lesions to the corresponding pattern of the adjacent normal tissue (Fig. 1). Evidence of MI was apparent in 6 (20%) cases (Table 1). The majority of the positive specimens (4 among 6) was affected in one marker while 2 specimens exhibited evidence of MI in 2 and 4 markers respectively. No association was found between the presence of MI and the type of the artery (aorta or basilar artery).

Recent data exist on several molecular alterations in atherosclerotic tissues, including abnormal expression of growth factors and cytokines (1,2). Furthermore, induction of apoptosis has been described and associated with the overexpression of programmed cell death-specific genes (23). At the DNA level the analysis is restricted to the detection of transforming oncogenes (5–7) and little is known of the genetic basis of the disease. In our study we describe an additional genetic alteration in atheromatous plaque DNA corresponding to an elevated mutational rate and affecting 20% of the specimens. Examining the specimens with additional markers might increase our figures. The precise significance of these findings remains obscure because the information as regards the genetic basis of the disease is limited. However, we may postulate that the relatively high mutational rate of the atherosclerotic lesions, as reflected in the instability of the microsatellite sequences indicates a destabilization of the genome which may affect other genes resulting in the disregulation of the cells harbouring these mutations. The latter may be associated with the proliferation of the smooth muscle cells (24) which is induced in the atherosclerotic lesions. This is also in agreement with the detection of transforming oncogenes in atherosclerotic plaques in several cases which is indirect evidence of a mutator phenotype.

It would be of interest to screen DNA repair genes for mutations in atherosclerotic plaques

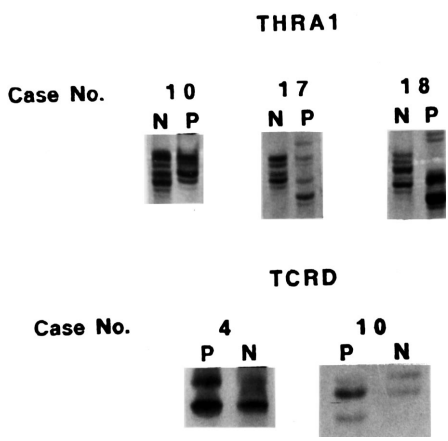

FIG. 1. Representative examples of specimens exhibiting MI. P corresponds to atherosclerotic plaque and N to adjacent normal tissue. In all cases a shift in the mobility of the microsatellites is obvious and thus the specimens were scored as positive for MI.

TABLE 1
Microsatellite Instability in 30 Atherosclerotic Lesions Tested with 7 Microsatellite Markers

Patient No.	D13S175	IFNA	TCRD	THRA1	HRM	D9S171	D19S49
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	−	−	−	−	−	−	−

exhibiting MI and investigate whether these mutations are also present in the germline of the patients. The latter may provide hints for the hereditary basis of the disease and is consistent with the observation that DNA repair deficiency may occur in phenotypically normal cells (25).

In summary, our report describes a novel molecular pathway for the development of atherosclerosis. We have detected a considerable incidence of MI in atheromatous lesions indicating that an elevated mutational rate is common in atheromatous tissues. MI is an early event in the DNA repair deficient-associated diseases. We suggest that MI, if it is indeed associated in the development of atherosclerotic plaques, is involved in the induction of the mitotic rate in smooth muscle cells. Future studies involving the evaluation of the clinical significance of this phenomenon as well as the molecular mechanism and consequences of MI may provide clues for the pathogenesis of the disease.

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