

## Microsatellite Instability Is Associated with Tumors That Characterize the Hereditary Non-Polyposis Colorectal Carcinoma Syndrome<sup>1</sup>

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### Abstract

Microsatellite instability implying multiple replication errors (RER+ phenotype) characterizes a proportion of colorectal carcinomas, particularly those from patients with the hereditary non-polyposis colorectal carcinoma syndrome. We studied the incidence of microsatellite instability in more than 500 sporadic tumors representing 6 different types of cancer. Apart from colorectal carcinoma [see the paper by Lothe *et al.* (Cancer Res., 53: 5849-5852, 1993)] the RER+ phenotype was found in 18% (6 of 33) of gastric carcinomas and 22% (4 of 18) of endometrial carcinomas. In contrast, no evidence of this abnormality was detected in cancers of the lung ( $N = 85$ ), breast ( $N = 84$ ), and testis ( $N = 86$ ). Importantly, the first three cancers, as opposed to the latter three, are characteristic of the hereditary non-polyposis colorectal carcinoma syndrome. These findings suggest that the cancers belonging to the hereditary non-polyposis colorectal carcinoma tumor spectrum may have essential pathogenetic steps in common, including a tendency to multiple replication errors.

### Introduction

Several cancers are thought to arise through a multistep process involving alterations of both tumor suppressor genes and proto-oncogenes. Evidence for a novel mechanism, a genome-wide tendency to replication errors, was recently found in a subset of colorectal carcinomas (1-3). Genomic instability, as demonstrated by shifts in the electrophoretic mobility of microsatellite repeat fragments, was shown to be particularly common (occurrence rate >70%) in colorectal carcinomas from verified HNPCC<sup>3</sup> patients (1). A gene predisposing to HNPCC has been mapped to chromosome 2p (4).

Apart from the colon, other organs, notably the endometrium and stomach, are frequent sites of cancer in HNPCC (5-7). On the other hand, there is no excess of laryngeal cancer, breast cancer, malignant brain tumors, or lung cancer in this syndrome (6). It has been argued that predisposition to HNPCC may even protect against lung cancer by a mechanism that is not known (6). There are no reports of testis cancer in the above cited large series of HNPCC cases.

In an attempt to shed light on the pathogenetic mechanisms of HNPCC we compared the incidence of the RER abnormality in cancers from six different organs. Since any gene that plays a role in a hereditary form of cancer is likely to be involved in a substantial proportion of sporadic cases of the same tumor type (8) we chose to

study a large collection of tumors mostly from sporadic cases. A striking association of the RER+ phenotype with tumors typical of HNPCC was found, implying similarities in the developmental pathways of these tumors.

### Materials and Methods

**Tissue Samples and DNA Extraction.** The analysis included 38 stomach cancers, 19 endometrial cancers, and 90 non-small cell lung carcinomas, and additionally, 252 colorectal carcinomas, 96 testicular germ-cell cancers, and 85 breast carcinomas described in detail in the paper of Lothe *et al.* (9). Paired normal and tumor samples were obtained from the patients after informed consent. The samples were from Norwegian patients except for stomach cancers that were from Portuguese patients.

DNA from blood was extracted by conventional methods (10). Tissue DNA was prepared from frozen biopsies (-70°C) except for colorectal cancer DNA that was prepared from single cell suspensions after nylon mesh filtration (11).

**Microsatellite Markers and RER Analyses.** Seven loci containing dinucleotide repeat sequences and representing different chromosomes (12) were studied in each case. The loci were (chromosomal localization): *D5S404* (5q); *D17S787* (17q); *D8S255* (8p); *D1S216* (1p); *D11S904* (11p); *D10S197* (10p); and *D13S175* (chromosome 13). In the study of lung cancers *D13S175* was replaced by *D3S1266* (3p). The markers were selected on the basis of two criteria: (a) the ability to combine primers so that all seven loci could be studied in only two polymerase chain reactions; and (b) chromosomal location; regions of both likely and unlikely involvement by other mechanisms, particularly LOH (13-17), should be represented.

The procedure for RER analysis was described in detail previously (4). Briefly, primers specific for each locus were used to amplify the repeat and short flanking sequences in template DNA by polymerase chain reaction. The products were labeled by [ $\alpha$ -<sup>32</sup>P]dCTP during the amplification reaction, followed by electrophoretic separation in 6% denaturing polyacrylamide gels and autoradiography.

### Results

RER+ tumors were defined in two alternative ways based on the number of affected loci (Table 1). Of the six tumor types studied, 10% of colorectal carcinomas, 18% of stomach carcinomas, and 22% of endometrial carcinomas were RER+ with two or more affected loci while breast, testis, and lung cancers were all RER- (Table 1). If tumors with microsatellite instability at only one locus were included the proportion of RER+ colorectal cancers increased to 17% (see a note for lung cancers below). The differences in the proportions of RER+ tumors between colorectal, stomach, and endometrial carcinomas were not statistically significant.

Examples of microsatellite repeat patterns in different extracolonic tumors are shown in Fig. 1. The observed mobility shifts resulted from 2-20 base pairs increases or decreases in fragment size. LOH or allelic imbalances occurred at similar overall rates in the different types of cancer studied (data not shown). All alterations observed in breast, testis, and lung cancers were compatible with LOH or allelic imbal-

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<sup>3</sup> The abbreviations used are: HNPCC, hereditary non-polyposis colorectal carcinoma; LOH, loss of heterozygosity; RER, replication error.

Table 1 *Microsatellite instability in different types of cancer*

| Organ                         | % of tumors showing the RER alteration/Locus |         |        |        |         |         |         | % of RER+ tumors (N) <sup>a</sup> with no. of affected loci |          |          |
|-------------------------------|--|---------|--------|--------|---------|---------|---------|---|----------|----------|
|                               | D5S404                                       | D17S787 | D8S255 | DIS216 | D11S904 | D10S197 | D13S175 | D3S1266   | ≥2       | ≥1       |
| Colon and rectum <sup>b</sup> | 8  | 8       | 5      | 5      | 7       | 7       | 10      |   | 10 (226) | 17 (243) |
| Stomach                       | 24   | 13      | 10     | 9      | 12      | 11      | 17      |   | 18 (33)  | 18 (33)  |
| Endometrium                   | 11   | 12      | 6      | 15     | 7       | 21      | 21      |   | 22 (18)  | 22 (18)  |
| Breast <sup>b</sup>           | 0  | 0       | 0      | 0      | 0       | 0       | 0       |   | 0 (84)   | 0 (84)   |
| Testis <sup>b</sup>           | 0  | 0       | 0      | 0      | 0       | 0       | 0       |   | 0 (86)   | 0 (86)   |
| Lung                          | 0  | 0       | 0      | 2      | 0       | 0       |         | 0   | 0 (85)   | 2 (87)   |

<sup>a</sup> N = number of scorable tumors, i.e., the added number of RER+ in that group and RER- tumors. In RER- cases information was obtained from 5 loci on the average (a successful study of a minimum of 3 loci was required and that should provide no evidence of the RER alteration).

<sup>b</sup> From the report of Lothe *et al.* (9).

ance (Fig. 1). The only exceptions were two lung cancers which showed an extra band at one locus (*DIS216*) each. Analysis of 4 more markers did not provide additional evidence of microsatellite instability in these cases.

## Discussion

The present microsatellite assays revealed alterations compatible with two different mechanisms, LOH and microsatellite instability. While LOH occurred at roughly similar rates in all cancers irrespective of the affected organ there was a marked organ specificity in the incidence of the RER abnormality in that a proportion of colorectal, endometrial, and stomach cancers were RER+ whereas all breast, testis, and lung cancers were RER-. Since clonality was equally present in the different tumors based on LOH it is likely that the basic pathogenetic mechanism is distinct in the RER+ tumors.

Recent evidence suggests that the tendency to form microsatellite alterations can be inherited and may be directly related to a gene causing susceptibility to a well-known familial cancer syndrome, HN-

PCC (1, 4). Interestingly, cancers that showed the RER abnormality in the present study are those commonly encountered in HNPCC patients while the others are not known to associate with HNPCC. It has been hypothesized that the HNPCC gene encodes a replication factor which, when defective, promotes genomic instability at several loci (1). The defective replication factor might affect target genes in different ways, depending on whether or not they contain mutation-prone sequences, particularly microsatellite repeats, at critical sites in their structure. The present findings suggest that the development of various cancers is regulated by genes that differ in their vulnerability to replication errors. This would explain why particular, single or multiple, organs are affected in a HNPCC patient who carries a mutant *HNPCC* gene in all his/her cells.

HNPCC is sometimes divided into two subcategories, Lynch syndrome I versus Lynch syndrome II, based on the absence vs. presence of extracolonic cancers (18). This distinction is not supported by the present RER findings. It is noteworthy that an ovarian cancer from a known HNPCC patient was previously shown to be RER+ (1) further questioning the basis of the subdivision. RER analysis may prove useful to define the HNPCC tumor spectrum in cases where epidemiological studies have yielded equivocal results. A good example is pancreas carcinoma which has been proposed to associate (19) or not to associate (20) with HNPCC.

The existence of a *HNPCC* susceptibility gene on chromosome 2p has been established by linkage but thus far all evidence regarding its possible mode of action is indirect. The cancer specificity of the microsatellite instability, as demonstrated by the present study, emphasizes the potential significance of this type of alteration in the pathogenesis of HNPCC. Further studies are in progress in our laboratories to determine the occurrence of microsatellite instability in cancers from verified HNPCC patients mainly by using paraffin-embedded tissue specimens.

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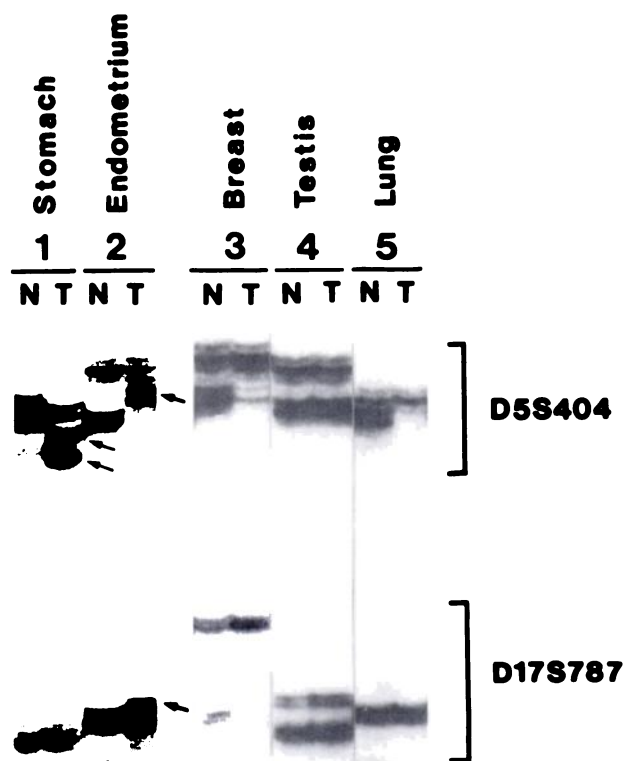


Fig. 1. Normal (N) and tumor (T) DNA samples from patients 1-5 with cancers of the indicated organs. Microsatellite repeat patterns at *D5S404* and *D17S787* are shown. Arrows, RER alterations. LOH is visible in tumor DNA from patients 3 (both loci) and 5 (*D5S404*).

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