Clinical usefulness of K-RAS mutation detection in colorectal cancer and in surgical margins of the colon

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Abstract

The incidence of K-RAS gene mutations in tumour and surgical margins was investigated in 63 patients with adenocarcinomas of varied clinical stage and histological grade. Point mutations of codon 12 K-RAS gene were detected, using the PCR-RFPL technique in cancer tissue in 23 patients (36.5%) and in colon margin mucosa in 1 patient (3.7%), out of 27 examined subjects. No significant correlations were found between the mutations and clinical features. Tumours, located in the left colon, and mucinous neoplasms displayed a higher incidence of mutations. No correlation was observed with either Dukes or TMN clinical advancement.

Key words: K-RAS mutations, colorectal cancer, surgical margins.

Introduction

Mutations of protooncogenes RAS are most commonly found in CRC, appearing early in the process of carcinogenesis, already in the phase of hyperproliferating epithelium, in anaplastic crypt foci (ACF), in adenocarcinomas and cancers [1, 2].

Determination of K-RAS mutations have recently been used to elucidate the prognostic role of surgical margin length during CRC resection, being more efficient than conventional histopathological examinations [3, 4, 5].

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The aim of the study was to clinically evaluate the usefulness of detection of K-RAS mutations in colorectal cancers and in surgical margins of the colon, with reference to their diagnostic and prognostic value in correlation with clinical features, histopathological picture, size, differentiation and metastases.

Material and Methods

The study involved 63 patients with CRC: 30 (47.6%) men, aged 51-80 years (the mean age: 68.0 years) and 33 women, aged 40-84 years (the mean age: 65.6 years) operated on during the period of 2001-2003. Tumours were located in the rectum in 30 patients, in the left colon in 19 and in the right colon in 14 subjects. In 25 cases, Dukes C tumours were found, 24 patients had Dukes B, 6 Dukes A and 8 Dukes D. Out of 61 patients with adenocarcinoma, 42 had G2 (68.8%), 14 G3 and 5 - G1 stage. In 7 patients, mucus-secreting tumours were isolated. Radical surgery was performed in 55 subjects (87.3%) from Groups A, B and C, palliative - in 8 D cases. Surgical margins of, at least, 5 cm in colon cancer and 2 cm in rectal cancer were preserved. The tumour and the margins were subjected to histopathological and molecular evaluation.

Genomic DNA was isolated from approximately 20 mg of tissue, using the GenElute Blood Genomic DNA Kit /Sigma/. The analysis of K-RAS mutation was performed by the RFLP-PCR technique with an additional enrichment of the genetic material with mutated alleles. The amplification proper (II PCR) and restrictive analysis with BstOI enzyme (sequences: KR1 5'-ACTGAATATAAACTTGTGGTAGTTGGACCT-3'KR2,5'-TCAAAGAAT-GGTCCTGCACC-3') followed the preliminary coping of the matrix. Digestion products were distributed, basing on differences in the molecular weight via electrophoresis in polyacrylamid gel. Electrophoregrams were subjected to computer analysis by UVI-KS400i/Image PC /Syngen Biotech. [6]. The chi2 test was used for statistical analysis: $p<0.05$ was the accepted level of significance.

Table 1. K-RAS mutations, according to age and sex of patients, tumour histological texture, histological and clinical advancement.

Feature	Number of patients	*K-RAS* mutation	p
Sex: Men	30	10 (33.3%)	
Women	33	13 (39.4%)	0.612
Age: under 60 years	17	6 (35.3%)	
over 60 years	46	17 (36.9%)	0.90
Tumour location: Rectum	30	10 (33.3%)	
Left colon	19	9 (47.3%)	
Right colon	14	4 (28.6%)	0.48
Dukes' scale: A	6	3 (50.0%)	
B	24	8 (33.3%)	
C	25	8 (32.0%)	
D	8	4 (50.0%)	0.98
Histological grade: G1	5	2 (40.0%)	
G2	42	15 (35.7%)	
G3	14	5 (35.7%)	0.98
Adenocarcinoma	56	19 (33.9%)	
Mucus-secreting tumours	7	4 (65.3%)	0.21
Feature T: pT1	6	3 (50.0%)	
pT2	6	2 (33.3%)	
pT3	37	13 (35.1%)	
pT4	14	5 (35.7%)	0.77

Results

K-RAS gene mutations in the study group of 63 patients were found in 23 cases (36.5%) in the tumour, in women 13/33 (39.4%) and in men - 10/30 (33.3%). Their incidence was similar in both age groups: under 60 in 6/17 patients (35.3%) and over 60 in 17/46 (36.9%). A higher percentage of mutations was observed in the tumours, located in the left colon: 47.3 and 28.6% (p=0.48) and in mucus-secreting tumours: 65.3% and 33.9% (p=0.2). No significant correlations were noted between Dukes scale and the tumour size (TNM) or histological differentiation (Table 1). K-RAS mutations were detected in 1 (3.7%), out of 27 surgical margins, with no neoplastic cells found in histopathological examination. In the group of 63 patients, a 6-24-month clinical follow-up revealed the disease recurrence in 5 (7.9%); two of them had K-RAS mutation in the primary tumour.

Discussion

Most clinical genetic researches tend to determine the prognostic significance of K-RAS mutations, but the reported data are not consistent [7, 8, 9]. The incidence of mutations in CRC ranges from 20% to 50% (the differences are due either to the selection of patients or to tissue fixation techniques), suggesting the existence of several different pathways of carcinogenesis which require thorough investigations [8, 9, 10]. In our material of 63 patients with colorectal cancer, mutations in codon 12 of K-RAS were found in 23 cases (36.5%). Similar results have been reported by other clinical centres [7, 9]. Like other authors, we found no correlation between the incidence of mutations and the clinical features, including clinical advancement [7, 8]. In our patients, a similar incidence of mutations was maintained from the earliest stages of tumour growth: 41.6% for T1 and T2 v. 35.2% for T3 and T4. No distinct correlations were seen between the presence of mutations in the tumour and its histological grade. Only in the mucus-secreting tumours, mutation incidence was close to significant 65.3% v. 33.9% (p=0.2). Similar correlations, resulting perhaps from a different genetic model of mucinous tumour growth, can be found in single reports of other authors [4, 9]. Neoplastic recurrence, following radical resection, was observed in 5 patients, out of 63 (7.9%), including 2 with gene K-RAS mutation. These observations, as well as the findings of other authors indicate that K-RAS mutations in CRC do not significantly correlate with either neoplastic recurrences or the survival time, although some contradictory data have been reported, e.g. the fact that either recurrences or remote metastases can be predicted, based on tests of tumour-drainage blood, bone marrow, lymph nodes, peripheral blood or stool [11, 12, 13]. Studies on K-RAS mutations are undertaken to detect micrometastases at the line of surgical incision of the colon margin. Their results (mutation range 6% - 53%) are difficult to compare because of the differences in the employed research models, in margin length and the methods of mutation detection [3, 4, 5]. In our own analysis of 27 margins >5 cm from the tumour border, mutation was detected in 1 (3.7%). The trials, which have been undertaken to determine K-RAS mutations in surgical margin mucosa, seem to confirm the possibility of their detection, when histopathological examination is negative. Determination of these mutations can be treated as one of the pathways, used to identify either high risk factors of micrometastases or recurrences in the postoperative period [5, 7, 10], what encourages research intensification.

Conclusions

No correlation was found of K-RAS gene mutations with clinical features of the tumour or with Dukes or TNM clinical advancement. A non-significant correlation was observed with the left-side location in the colon and with mucinous histotype.

In histologically unchanged mucosa of surgical margins, K-RAS point mutations were detected in 3.7% of CRC patients.

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