Detection of Mutant K-*RAS* Sequences in the Urine of Cancer Patients

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DNA fragments from apoptotic cells crossing the renal barrier retain their matrix functions, which allows PCR identification of mutant sequences in excreted DNA. We investigated the possibility of detecting k-*ras* mutations in urinary DNA of tumor patients (colon cancer). In some patients with k-*ras* codon 12 mutations in tumor cell DNA the same changes were detected in the urinary DNA. The possibility of using this approach for early diagnosis and monitoring of tumors is discussed.

Key Words: *cancer; k-ras oncogene; apoptosis, urine*

Previous animal experiments demonstrated that DNA fragments of several hundreds b.p. originating from apoptotic cells can cross the renal barrier and retain their matrix functions in PCR [2]. This conclusion was extrapolated to humans, as it was possible to identify sequences originating from human internal media in the urinary DNA. For instance, sequences specific for masculine Y chromosome were detected in the urine of women after blood transfusion from male donors and in pregnant women with male fetuses [2]. In some patients with k-*ras* codon 12 mutations in the tumor tissue the same changes were identified in the urinary DNA. Our present and previous [1,4] studies demonstrate the possibility of PCR analysis of urinary DNA for diagnosis and monitoring of tumor growth. Recent successful attempts at detection of mutant sequences originating from the tumor in the serum and plasma of cancer patients confirm this possibility [3,5-13]. Here we evaluated the possibility of detecting mutant k-*ras* in urinary DNA of patients with colon cancer (these mutations are most typical for this tumor).

MATERIALS AND METHODS

Fifteen patients with colon adenocarcinoma of different degree of differentiation treated at the Proctology Department of the Institute of Clinical Oncology, Cancer Research Center, were examined.

The sources of DNA were urine, tumor tissue , and adjacent normal mucosa obtained during surgery. DNA was isolated from tissues by the standard phenol method.

Before surgery, 25-50 ml fresh (not morning) urine was collected into a sterile container with 5 ml 250 mM EDTA for blocking DNAse activity. DNA was isolated by 6 M guanidine isothiocyanate treatment followed by adsorption on columns with glass powder Wizard Minipreps DNA purification system (Promega). The columns were washed with 80% isopropanol and DNA was desorbed with a low ionic strength solution as recommended by manufacturer.

PCRwas carried out in a standard buffer (50 μl). DNA (30-50 ng) isolated from tumor tissue or normal mucosa or 5 μl DNA from the urine was added to the

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reaction mixture. k-*ras* codon 12 mutations were detected by the so-called "enriched PCR" [9]. The method consists in the creation (by means of modified primers) of two artificial *Bst*NI restriction sites in the wild type sequence and one in the mutant sequence. Intermediate stage of *Bst*NI restriction eleminates wild type sequences and the samples become enriched with mutant sequences. After 30-35 additional PCR cycles and *Bst*NI treatment of reaction products, electrophoresis in 10% PAAG was carried out. DNA was stained with ethidium bromide (0.5 μg/ml). The mutant sequence was detected as a 142 b. p. band and wild type sequence as a 113 b. p. band. The original PCR product not treated with restrictase yields a 157 b. p band.

DNA from SW480 cell with one deleted k-*ras* allele and one allele with codon 12 mutation was used as the positive control.

RESULTS

Gene k-*ras* codon 12 mutations were detected in 9 of 15 patients with colon adenocarcinoma, which is in line with published reports on high incidence of activation of this oncogene in colon tumors [6,9]. The same mutation was detected in urinary DNA from 4 of 9 patients with activated k-*ras* in tumors. Figure 1, *a* shows representative results of analysis for one of these patients. Intensive band corresponding to mutant sequences is present in tumor DNA (runs 2) and a less intensive but clearly seen in urinary DNA (runs 3). Hardly discernible band corresponding to mutant sequence in DNA from normal mucosa adjacent to the tumor (runs 1) can be due to the presence of some tumor cells.

No k-*ras* mutations were detected in tumor tissue and urine of 6 patients (Fig. 1, *b*). No mutations in urinary DNA were detected in 9 healthy donors.

Our findings indicate the possibility of detecting tumors growing in the organism by detection of specific mutant sequences in urinary DNA. Improvement of method sensitivity by using radioactive or fluorescent label in the PCR analysis can essentially improve reliability of detecting tumor cells in the organism. The potentialities of this approach as a new method for early tumor diagnosis and monitoring is now investigated.

The advantages of detecting mutant sequences in urinary DNA over recently developed analysis of plasma or serum DNA [3,5-13] are obvious: it is noninvasive, their is no risk of infection with HIV or other pathogens, DNA can be isolated from greater volumes of initial material and is easier to isolate due to low concentration of proteins (1000-fold lower protein content); moreover, urine contains no PCR inhibitors present in human blood.

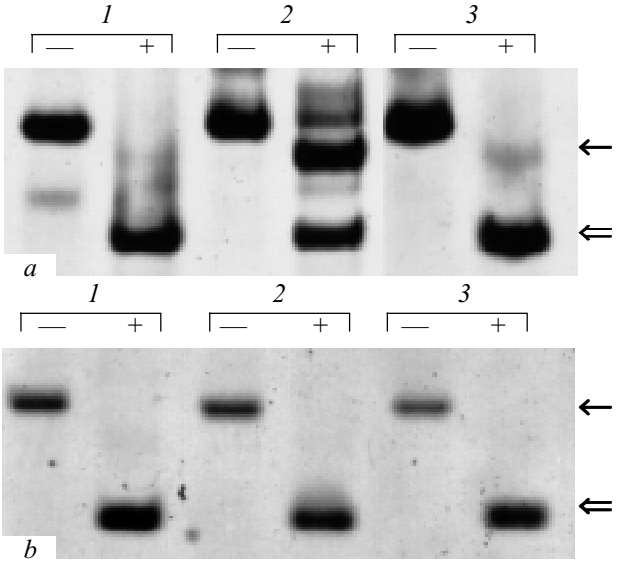

Fig. 1. Detection of k-*ras* codon 12 mutations in patients with colon cancer. *1*) DNA of normal mucosa adjacent to tumor; *2*) tumor DNA; *3*) urinary DNA. Sequences amplified by PCR before (-) and after (+) treatment with *Bst*NI are presented. Arrows show mutant sequence, double arrows wild-type sequences. *a*) patient with k-*ras* mutation in tumor and urinary DNA; *b*) patient without k-*ras* mutations in urinary and tumor DNA.

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