Effect of tumor removal on mucosal protein synthesis in patients with colorectal cancer

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Rittler, Peter, Hans Demmelmaier, Berthold Koletzko, Karl-Walter Jauch, and Wolfgang H. Hartl. Effect of tumor removal on mucosal protein synthesis in patients with colorectal cancer. Am J Physiol Endocrinol Metab 284: E1018–E1021, 2003. First published January 21, 2003; 10.1152/ajpendo.00474.2002.—It is currently controversial whether mucosal hyperproliferation is involved in colorectal cancerogenesis. The purpose of the present study was to examine protein synthetic rate as an indicator of potential tissue proliferation in grossly normal rectal mucosa from cancer-bearing subjects and to compare this rate with that in mucosa from subjects posttumor removal. Six postsurgical control subjects with localized rectal cancer and five postsurgical control subjects received a primed constant infusion of [1-13C]leucine (0.16 μmol/kg min, 9.6 μmol/kg prime). Forcible biopsies from the mucosa were taken after 3 and 6 h. Protein synthesis was calculated from protein-bound leucine enrichment (determined by capillary GC-combustion IRMS) and from the enrichment of free intracellular leucine (determined by GC-quadrupole MS). In cancer-bearing subjects, mucosal protein synthesis amounted to 1.28 ± 0.24%/h. This rate was significantly higher (P < 0.05) than the corresponding rate of mucosa from patients after cancer removal (0.69 ± 0.09%/h). These findings do not support the concept that colorectal cancer originates from a proliferative disease of the whole colon. Increased mucosal protein synthesis appears to depend on the presence of the tumor itself and should therefore be considered a secondary phenomenon.

colorectal mucosa; protein synthesis; stable isotopes

THERE IS AN ONGOING QUESTION whether colorectal cancer should be considered a local disease or rather as a uniform organ disease that is not only localized at an individual site along the colorectum. The hypothesis that was originally suggested for the transition from normal mucosa to adenoma involved three steps (5). At stage I, the proliferative compartment of the colorectal crypt, which is usually confined to the lower one-third of the crypt, extends upward and envelops the entire crypt. At stage II, the maximum of the proliferative compartment shifts to the upper portion of the crypt, and, at stage III, the total number of replicating cells in the crypt rises, leading to mucosal hyperproliferation and subsequently, because of the influence of cofactors, to neoplastic transformations (adenoma). However, in patients with sporadic colorectal cancer, findings are controversial, and the scientific literature is divided into reports supporting (2, 18, 20, 23, 26) or contesting (1, 4, 15, 21, 22, 27) the importance of the stage III defect for adenoma and carcinoma formation.

A possible explanation for this discrepancy in patients with sporadic colorectal cancer might be found in the techniques used to evaluate mucosal proliferation. Most of these methods are applied in vitro and are cytostatic, only providing an estimate of the fraction of proliferating cells in a particular phase of the cell cycle at a given moment. This information can be misleading, since it uses no measure of time (length of the cell cycle). Duration of the cell cycle may, on the one hand, be a major component of tissue proliferation and growth but is, on the other, extremely difficult to examine in vivo because of the associated invasive measures (8).

We have recently developed a technique that enabled us to perform dynamic proliferation measurements in vivo independent from surgery or anesthesia. This minimally invasive technique is based on advanced stable isotope methodology and focuses on the determination of the fractional protein synthetic rate as an indicator of tissue proliferation (9). In the present study, we investigated the effect of tumor removal on mucosa protein synthetic rates in situ in patients with sporadic colorectal tumors. Tumor removal should not affect mucosal protein synthesis if such a phenomenon would indeed indicate a uniform organ disease associated with colorectal carcinogenesis.

MATERIALS AND METHODS

Patients. Two groups of subjects, one with locally limited rectal carcinoma (n = 6, 3 males, 3 females), the other after curative surgery for colorectal cancer (control group; n = 5, 3 males, 2 females), were studied. The groups were comparable in age (carcinoma: 67.3 ± 5.0 yr; control: 64.5 ± 5.2), body weight (carcinoma: 70.0 ± 5.7 kg; control: 66.0 ± 4.2), height (carcinoma: 168 ± 3 cm; control: 165 ± 2), and body mass index (carcinoma: 25.0 ± 2.7 kg/cm²; control: 24.3 ± 1.7). No
subject had a history of previous weight loss or clinical and laboratory signs of malnutrition or metabolic diseases.

In cancer patients, previous tumor biopsies and clinical staging examinations (abdominal computed tomography scan, colonoscopy, chest X-ray) had demonstrated adenocarcinoma without evidence for gross local spread or distant metastasis (preoperative staging T1 or T2, N0 or N1, M0). Histopathological examinations had shown moderate or poor differentiation in all cases. Details and data from cancer patients were already presented in a previous publication (9). Control subjects were studied before the study, the subjects remained postabsorptive, except for consumption of mineral water. A primed constant infusion rate of 0.16 kcal/kg day, including 20 g/kg of amino acids from proteins were then converted to the plasma leucine. Plasma background enrichment was shown to reflect both intracellular protein-bound and free leucine background enrichment (9). The first biopsy was performed after 180 min of isotope infusion, and the second was performed after 360 min. Forceps mucosa biopsies were all taken with the patient awake and supine from the rectosigmoidal transition zone. No narcotics or agents for bowel preparation were used. The minimum distance of mucosa biopsy site from the tumor was 5 cm.

Procedures. The principles of the method and the generation of the data are presented and discussed in detail in a previous publication (9). The free and protein-bound amino acids in tissue biopsies were separated by precipitation of the amino acid-NAP-amino acid derivatives were analyzed in a capillary GC-combustion IRMS analysis, amino acids from proteins were then converted to the N-acetyl-n-propyl (NAP) ester. For GC-quadrupole MS analysis, we prepared the tert-butyldimethylsilyl (t-BDMS) derivative from free intracellular amino acids. NAP-amino acid derivatives were analyzed in a capillary GC-combustion IRMS system that consisted of a Hewlett-Packard 5890 series II gas chromatograph that was interfaced to a mass spectrometer Delta S (Finnigan MAT, Bremen, Germany). t-BDMS derivatives were analyzed by a GC-MS system (MSD 5971D; Hewlett-Packard). Isotopomer ratios of the sample were obtained by electron-impact ionization and selected ion monitoring at mass-to-charge ratios 303 and 302. Data were expressed as tracer-to-tracee ratios.

Calculations. The tissue fractional synthetic rate was calculated by dividing the increment in the protein-bound [1-13C]leucine tracer-to-tracee ratio by the enrichment of the precursor, the free intracellular [1-13C]leucine tracer-to-tracee ratio. Delta increments of protein-bound [1-13C]-leucine enrichment between biopsies were calculated from isotope ratios [13C]/[12C] using a correction factor that takes into account dilution of the label at the carboxyl position by the other carbon atoms in the derivatized leucine (9). Fractional synthetic rate (FSR) was then calculated as follows:

\[
FSR = \frac{RL(t_2) - RL(t_1)}{(RL(t_2) + RL(t_1))/2} \times \frac{1}{\Delta t} \times 60 \times 100 \text{ (%/h)}
\]

RL(t1) and RL(t2) correspond to the tracer-to-tracee ratio of tissue protein-bound leucine in two subsequent samples (1 and 2), separated by the time interval \(\Delta t\). RL(t1) and RL(t2) indicate the tracer-to-tracee ratios of mucosa-free leucine in two subsequent samples. Average values between RL(t1) and RL(t2) were used as precursor enrichments for mucosa protein synthesis. The factors 60 (min) and 100 are needed to express the fractional synthetic rate in percent per hour.

Statistics. Data are expressed as means ± SE. Interindividually comparisons were made by the unpaired t-test. A P value <0.05 was taken as indicating a significant difference.

RESULTS

The tracer-to-tracee values of [1-13C]leucine in tissue protein and in the free, intracellular amino acid pool at the two sampling points are presented in Table 1.

Calculation of mucosa fractional synthetic rate in tumor-bearing subjects amounted to 1.29 ± 0.28%/h. The mucosa fractional synthetic rate after tumor removal (0.69 ± 0.09%/h) was significantly lower (P < 0.05).

DISCUSSION

The approach to study tissue proliferation by measuring protein synthetic rate is based on cell experiments demonstrating a direct correlation between the rate of cellular reproduction and of cellular protein synthesis. Experiments in transformed cells could show that an increase of protein synthetic rate accompanies cell growth (11). Consequently, artificial interruption of protein synthesis arrests cellular prolifera-

Table 1. Tracer-to-tracee ratios of [1-13C]leucine in mucosa protein and in the free, intracellular amino acid pool

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>AA</td>
</tr>
<tr>
<td>Control Mucosa</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td>5</td>
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<tr>
<td>Unaffected Mucosa</td>
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</tr>
<tr>
<td></td>
<td>2</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
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<td>0.28</td>
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<td></td>
<td>6</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Units are %. P, protein; AA, amino acid. Sample 1 was taken after 3 h and sample 2 after 6 h of isotope infusion.
MUCOSAL PROTEIN SYNTHESIS IN COLORECTAL CANCER

likely that patients with sporadic colorectal cancer subjects is secondary to the presence of the tumor itself. The operation of grossly normal mucosa in tumor-bearing subjects is without a personal or family history of cancer (21, 22). The latter studies suggest that mucosal fractional protein synthetic rates posttumor removal are normal. However, in these studies, mucosal proliferation was determined in vitro via [3H]thymidine or 5-bromo-2-deoxyuridine incorporation. These methods are cytostatic and do not account for the speed of cellular proliferation (which means the duration of the cell cycle) but measure only the fraction of proliferating cells. Therefore, corresponding results may not necessarily reflect the in vivo situation where the length of the cell cycle is also a major component of mucosal growth. On the other hand, determination of mucosal protein synthesis in vivo represents a dynamic method that (in the gastrointestinal tract) combines all aspects of tissue growth. Consequently, mucosal protein synthesis is commonly believed to reflect largely enteroxyte proliferation (10, 13).

In contrast to the above in vitro results, we compared mucosal protein synthesis after tumor removal with that of tumor-bearing subjects in vivo. Therefore, our data add much stronger and direct support to the hypothesis that tumor-associated mucosal hyperproliferation of grossly normal mucosa in tumor-bearing subjects is secondary to the presence of the tumor itself and disappears after tumor removal. Thus it is unlikely that patients with sporadic colorectal cancer suffer from a generalized disease (stage III defect) that can be demonstrated in the colorectum at a significant distance from the tumor. In the latter case, local tumor removal would not have affected large bowel protein synthesis. However, our technique does not allow exact determination of the underlying mechanisms causing the increased rate of protein synthesis in cancer patients. In theory, such a change may result from either an increased number of proliferating cells or an acceleration of the cell cycle, or from both mechanisms.

It is a matter of controversy whether grossly normal mucosa of patients with sporadic colorectal cancer demonstrates an increased number of proliferating cells. Thus some authors reported an increased percentage of cells in the S- or M-phase in seemingly normal mucosa of patients with sporadic colorectal cancer when 5-bromodeoxyuridine or [3H]thymidine incorporation was determined (2, 16, 18, 23, 26) or when mitoses were counted (20). On the other hand, using the same or similar (PCNA staining) histochemical methods, other investigators were unable to demonstrate such significant differences between mucosa from patients with colorectal cancer and that from healthy subjects (4, 15, 27). Therefore, it is possible that, besides a growing number of proliferating cells, also an accelerated cell cycle contributed to the increased protein synthetic rate and thus to the hypoproliferative state of grossly normal mucosa in cancer-bearing patients.

Irrespective of the underlying local mechanism, the presence of a colorectal carcinoma may stimulate intestinal proliferation and protein synthesis at remote sites in two ways. First, colorectal malignomas were found to release a variety of growth factors, such as transforming and vascular endothelial growth factors (16), which may ultimately find their way into the systemic circulation (6, 7). Since these growth factors are potent stimulators of normal intestinal epithelial proliferation (14), they could well be responsible for the increased protein synthetic rate found in uninvolved mucosa of cancer-bearing subjects. Second, there is evidence that carcinoma may trigger immunological reactions in the host, which include a systemic release of cytokines (3). Cytokines were recently found to be potent stimulators of intestinal proliferation (24, 25) and could, therefore, also explain the tumor-related rise in mucosa protein synthesis.

Our data suggest that mucosal hyperproliferation in cancer-bearing subjects appears to be a secondary phenomenon and not a sign of a uniform organ disease or of a premalignant mucosal change.

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REFERENCES


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