Short Communication

Transforming Growth Factor α Distribution in Rectal Crypts As a Biomarker of Decreased Colon Cancer Risk in Patients Consuming Cellulose

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Abstract

Data from rat experimental carcinogenesis studies indicate that supplemental dietary cellulose reduces the incidence of colon cancer. Epidemiology studies also indicate that high dietary fiber reduces the risk of colorectal cancer in humans. Patients diagnosed with sporadic adenomas were entered into a randomized clinical trial to determine if supplemental dietary cellulose would reduce the patients’ risk for colon cancer. Immunohistochemical staining for transforming growth factor α (TGF-α) was done on biopsies of rectal mucosa taken from patients at the time of initial polypectomy and 1 year later. Results were evaluated for utility as a surrogate end point biomarker for reduction in colon cancer risk. There was a significant decrease in the fraction of the rectal crypt cells that stained for TGF-α in six of seven of the patients given the cellulose supplements but in only one of six of the patients not given cellulose. Thus, whether evaluated as a group or in individual patients, there was a significant decrease in TGF-α in rectal crypts due to cellulose intervention, which correlated with the expected ability of supplemental dietary cellulose to decrease the risk for colon cancer. Long-term testing of the ability of dietary cellulose to reduce adenoma recurrence is under way to validate the use of TGF-α as a surrogate end point biomarker.

Introduction

The results of animal studies have shown that carcinogen-induced colon cancer may be prevented by various dietary changes or chemical interventions (1-7). In animal studies, alteration in various morphokinetic parameters in the colonic epithelial crypts (i.e., index of cell proliferation, distribution of proliferating cells, or the size of the proliferative zone) has been demonstrated to correlate with alteration in colon cancer risk (1, 2, 8). Thus, alteration in these morphokinetic parameters has been proposed as a possible surrogate end point (SEB) for evaluating the efficacy of chemopreventive interventions in humans at higher than normal risk for colorectal cancer (8-11). However, using proliferation markers (as determined by proliferating cell nuclear antigen or [3H]thymidine) as SEBs for judging the efficacy of an intervention in human trials has proved less reliable than in animal studies (12-16); thus, other SEBs are needed for use in short-term chemoprevention trials.

Preliminary studies in this laboratory indicated that the pattern of TGF-α staining in the crypts of rectal mucosal biopsies might be used as a quantifiable SEB. TGF-α is normally localized in the upper one-third to two-thirds of “normal” human colonic crypts (17). The expression of TGF-α is often increased in colonic adenomas and adenocarcinomas (18). It is not yet known if the expression of TGF-α is different in a rectal biopsy of normal-appearing mucosa from patients at high risk than in those at less risk for developing a colon adenocarcinoma.

We are currently conducting a long-term randomized dietary intervention study to reduce the risk for colon cancer in patients who are postpolypectomy of a confirmed adenoma. The intervention consists of supplementation of the diet with 15 or 25 g of microcrystalline cellulose per day. The results of animal studies have shown that increased dietary fiber in the form of cellulose will significantly decrease the risk for colon cancer (1-3, 19-21). We report here that there was a significant decrease in the extent of TGF-α staining in the colonic crypts of six of seven individual patients who consumed cellulose. Only one of the seven patients who did not consume the cellulose supplement had significantly decreased TGF-α staining, whereas two other patients who did not consume cellulose supplement actually had a significant increase in the extent of TGF-α staining. Thus, in most of the individual patients and in the group of patients that consumed dietary cellulose supplementation, a decrease in the extent of TGF-α staining correlated with the expected ability of supplemental dietary cellulose to reduce the risk for developing colon cancer.

Materials and Methods

Patients. The primary study is a multicenter, prospective, randomized, control trial using patients who have developed a sporadic adenoma. Patients are recruited from Audie Murphy Memorial Veterans Hospital, Wilford Hall United States Air

1 Received 1/14/97; revised 4/15/97; accepted 4/21/97.

3 The abbreviations used are: SEB, surrogate end point biomarker; TGF, transforming growth factor.
The aid of a stereo dissecting microscope so that the biopsy could be placed into the paraffin block "on-edge" and midaxial longitudinal crypts would be obtained on the microscope slides after the sections were cut. All biopsies obtained at one time from a single patient were placed into the same cassette for paraffin embedding. Following paraffin embedding, 4-μm-thick sections were cut, and three sections from each block were placed on coated slides (Plus-coated microscope slides; Fisher Scientific). Laboratory personnel were blinded to the treatment group of the patient until completion of all immunohistochemical data collections and statistical evaluations of the individual data.

**Immunohistochemistry.** Routine immunohistochemical techniques were used to localize TGF-α proteins on the deparaffinized tissue sections. TGF-α primary antibody (Ab-2, clone 231–4.4, 1:10 dilution) was from Oncogene Science (Cambridge, MA). Secondary antibodies [Super Sensitive link (anti-IgG-biotin) and label (avidin-horseshad peroxidase)] and liquid diaminobenzidine were from Biogenex (San Ramon, CA). A single primary antibody was applied to two tissue sections on each slide. The third tissue section on each slide was used as a negative control, with normal mouse serum substituted for the primary antibody and incubated with all other reagents.

**Calculation of TGF-α Parameters.** Only complete midaxial, longitudinally sectioned crypts were selected for determining the extent of TGF-α stain. The stringent criteria used to identify countable crypts were: (a) base of the crypt immediately adjacent to the muscularis mucosa; (b) a single column of cells visible from the base of the crypt to the mouth of the crypt; (c) lumen of the crypt visible from the base to the mouth; and (d) surface epithelium present.

In typical rectal biopsies, the nucleus and cytoplasm of mucosal epithelial cells stain positive for TGF-α for about one-third to one-half of the distance from the mouth to the base of the crypt (Fig. 1). Further toward the base of the crypt, a few nuclei may stain intensely positive. In some crypts, a few cells at the very base of the crypt showed positive nuclear and cytoplasmic staining. Parameters recorded for TGF-α staining were: (a) total crypt height in number of cells; and (b) number of cells that exhibited positive stain in a continuous column from the mouth down the side of the crypt. Solitary TGF-α-positive cells and cells at the bottom of the crypt were not included in the count. The fraction of the crypt that was stained was calculated as the number of cells with cytoplasmic and nuclear stain (counted from the top down) divided by the crypt height in number of cells. The entire protocol for the use of TGF-α as a biomarker has a patent pending.

**Statistical Analyses.** SAS for the PC was used for statistical analyses. Data from slides taken at each biopsy time for each individual were subjected to paired r tests to determine if there were differences in the extent of TGF-α between the first and second biopsy for the individual. The mean extent of TGF-α stain from each individual in each group was then used in a statistical analysis to find differences between groups which did or did not consume cellulose or between the first and second biopsy of a group; significance was accepted with a probability value of P ≤ 0.05. A nonparametric comparison of two proportions was used to indicate whether a greater proportion of patients that received cellulose had significantly decreased TGF-α staining in the colonic crypts compared to the group that did not receive cellulose.

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4 W.E. Hardman and I.L. Cameron. Type of chemical fixative effects the immunohistochemical localization of TGFα in rat colon crypts, manuscript in preparation.
Comparison of the proportion of each group that showed a significant decrease in the percentage of the crypt that stained positive for TGF-α (six of seven in the group that consumed cellulose supplement versus one of six in the group that did not consume cellulose) showed a significant difference between groups ($P = 0.05$). These results indicate that consumption of cellulose significantly altered the localization of TGF-α within the colonic crypt.

Discussion

Dietary modification and chemoprevention measures have been proposed as practical means to reduce morbidity and mortality from colon cancer (22). The results of animal studies have identified a number of agents that may be useful colon cancer preventives (23, 24). Unfortunately, in humans, the high expense, the large number of patients required, and the long time necessary to carry trials of potential chemopreventive agents to the end point of cancer incidence limit the number of agents that can be tested for their effect on colon cancer incidence.

Identification of SEBs, which are altered early in the process of carcinogenesis and which are altered by the putative preventive agent, would speed the process of identification of effective cancer preventive interventions in humans. To be useful, SEBs: (a) should be either on or closely associated with the pathway to colon cancer so that alteration in the biomarker correlates highly with alteration in cancer risk; (b) should be modified from “normal” in patients at high risk for colon cancer; (c) should be capable of being shifted from “normal” by the intervention in a relatively short time; and (d) must be validated as being predictive of alteration in colon cancer risk (25). The development of SEBs that can be modified in a predictable direction in short-term studies in humans would reduce the cost of chemopreventive trials and strengthen the rational for longer term studies on promising cancer preventive measures (23, 26, 27). An SEB that shows that the intervention was effective at reducing the risk for colon cancer in an individual patient would be an especially valuable SEB because it could serve to provide the patient encouragement to incorporate a long-term intervention into his/her lifestyle to reduce the risk of developing colon cancer. The extent of TGF-α stain in colonic crypts appears to meet most, if not all, of the requirements for a useful SEB.

TGF-α. The growth factor TGF-α is a $M_r 5,000$ to $M_r 20,000$ secreted protein (28) that was first identified in the culture of cells that were transformed by retroviruses (29). The active form of TGF-α binds to the epidermal growth factor receptor and is reported to stimulate proliferation of epithelial cells in vitro (30–32). TGF-α is produced by normal colonic mucosal epithelium and is found in the epithelial cells located in the upper one-third to two-thirds of the normal human colonic crypt (17, 32). Little is known about the precise regulation or activity of TGF-α in the normal colonic epithelium. However, it is known that TGF-α is a mitogen for colonic epithelial cells in vitro (30) and that the mitogenic activity of TGF-α can be blocked by either TGF-α antisense constructs or by antibodies to the epidermal growth factor receptor (32–34). Recent studies have proposed that TGF-α also has a role in epithelial cell differentiation (17, 35, 36). TGF-α is reported to be overexpressed in about 24% of colon adenomas and 81% of colon adenocarcinomas (18) and has been identified at higher levels in the serum of patients with gastrointestinal cancer than in patients without cancer (37). TGF-α can be induced in vivo and in vitro by deoxycholate, a colon tumor-promoting bile acid (38). The findings listed above and the results from a study...
The first rectal biopsy from each patient was obtained at the time of polypectomy; the second biopsy was obtained 1 year later. Dietary cellulose-supplemented patients consumed 15–25 g of microcrystalline cellulose daily. Values given are the fraction of cells in the crypt column, stained from the top down, which was positive for TGF-α. Solitary TGF-α-positive cells and cells at the bottom of the crypt were not included in the count. A complete description of the procedure is given in "Methods and Materials."

<table>
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<th>First biopsy</th>
<th>Second biopsy</th>
<th>P* Direction of change</th>
<th>% change</th>
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<tr>
<td>n* Fraction stained ± SE</td>
<td>n Fraction stained ± SE</td>
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<td>With cellulose supplement</td>
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<td>6</td>
<td>0.25 ± 0.04</td>
<td>7</td>
<td>0.31 ± 0.030</td>
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<td>Without cellulose supplement</td>
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* n, number of crypts counted per patient.
* P < 0.05 indicates a statistically significant change in TGF-α distribution in an individual patient.

Fig. 2. Fig. 2 graphically illustrates the data in Table 1. The fraction of the cells in the crypt column, from the mouth down, which was positive for TGF-α was significantly decreased in six of seven patients in the group that consumed 15–25 g daily of dietary cellulose supplement and was significantly decreased in only one of six patients that did not consume cellulose. The first biopsy was taken at the time of polypectomy; the second biopsy was taken 1 year later.

showing that the overproduction of TGF-α in breast epithelium was promotional to breast cancer formation (39) support the hypothesis that TGF-α has a role in tumor promotion. Given that TGF-α is an endogenously produced growth factor that is promotional for colon cancer, then one might expect that immunohistochemical evidence of a decrease in TGF-α in colorectal mucosa could serve as a biomarker of reduced risk of colon cancer.

The results from this study are particularly encouraging for the use of TGF-α as a biomarker of alteration in colon cancer risk. It was possible to detect significant differences in alteration in TGF-α between the intervention group of patients and the nonintervention group of patients despite the small number of subjects per group. Even more important, there was good precision at determining differences in the extent of TGF-α positively stained cells in the colonic crypt before and after the intervention in individual patients, even when few crypts could be counted. Additional work still remains to be done to establish TGF-α distribution in rectal mucosal crypts of patients as a useful and well-understood SEB of colon cancer risk including: (a) defining the role of TGF-α in the colon; (b) uncovering the molecular pathways that may lead to suppression of TGF-α expression; (c) validating that alteration in the biomarker is predictive of alteration in colon cancer risk; and (d) testing use of TGF-α as a biomarker for other chemopreventive interventions.

Acknowledgments

We gratefully acknowledge the FMC Corporation for the gift of the microcrystalline cellulose.

References


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