Nuclear Size and Nuclear Binding of Tritiated Actinomycin D into Epithelial Cells of Colon Cancer Patients with Apparently Normal Colorectal Mucosa¹

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ABSTRACT

The nuclear binding of tritiated actinomycin (³⁵H)AM) is clearly linked to the structural organization of the chromatin. (³⁵H)AM decreases in cells which differentiate and becomes very low in fully differentiated cells. (³⁵H)AM nuclear binding and nuclear size were concomitantly measured in normal-appearing flat mucosa of 10 patients bearing a colorectal cancer and compared with the colon mucosa of 10 normal individuals. In the colon of these normal subjects, there is a decreasing gradient of labeling in the upper third of the gland, with heavy labeling in the lower two-thirds and light labeling in surface epithelial cells. The ratio (R) of grain count in cells in the bottom of the glands to grain count in the surface epithelial cell varies in normal subjects from 4 to 14. There is no correlation between nuclear size and (³⁵H)AM binding. In normal-appearing flat mucosa of cancerous patients, there is no decrease of labeling (³⁵H)AM in the upper third of the glands; superficial cells are as labeled as those at the bottom (R = 1). There is a correlation between nuclear size and (³⁵H)AM in these cells. Normal-appearing cells of colorectal cancer patients are probably not involved in a normal process of differentiation.

INTRODUCTION

The proliferative compartment is localized in the lower two-thirds of crypts in the normal human colon. This has been extensively demonstrated by incorporation of (³⁵H)thymidine. An extension of the proliferative compartment to the upper portion of the glands has been demonstrated in the mucosa of isolated adenoma itself (16), in the mucosa of patients with "familial polyposis" (5, 15), in patients with previous polyps (16) or colon cancer (23), and in the colons of dimethyldrazine-treated mice (17). In patients with previous colon cancer, this abnormality seems to be patchy (23). Small focal areas of hyperplasia (10, 18, 22, 27, 29, 30, 34) or of epithelial abnormalities (2) may be found in the mucosa of the large intestine in the vicinity of the carcinoma. Filipe and Branfoot (18) and Riddell and Levin (27) have described "transitional" mucosa both confined to the carcinoma and existing in patches away from the tumor. This "transitional" mucosa demonstrates an alteration in the relative proportions of the different cell types along the crypts, an increase in the number and size of goblet cells, and hyperplasia. These changes could be due to a loss of normal differentiation. The reports of Filipe and Branfoot (18) and Riddell and Levin (27) suggest that the changes in transitional mucosa represent a specific premalignant alteration in colonic mucosa. However, Isaacson and Attwood (20) suggest that these alterations are secondary phenomena because they are also observed in conditions such as solitary ulcer syndrome and squamous cell carcinoma of the anus. We have studied nuclear binding of (³⁵H)AM in the colon mucosa of normal individuals and have compared it with the apparently normal colonic mucosa of patients with concomitant colon cancer. Actinomycin D is a cytotoxic drug which binds specifically to DNA. When tritiated, it is possible to detect the presence of actinomycin D and to quantify it in autoradiographic preparations of histological sections of tissues. It has been shown previously that the quantity of bound (³⁵H)AM is lower in differentiating than in dividing cells (9), is restricted by chromosomal proteins (3, 4, 11, 12, 24, 28), decreases in cells which differentiate, and becomes very low in fully differentiated cells (8, 26, 31, 32).

MATERIALS AND METHODS

The patients were prepared for colonoscopy and biopsy by being placed for a prescribed period on a low-roughage diet followed later by 2 mild enemas. Tissue samples were fixed in pure methanol for a 24-hr period and then processed through to paraffin. Sections 3 μm thick were obtained, and those used for routine histopathological diagnosis were stained with hematoxylin and eosin. Biopsies were taken from normal-appearing mucosa of 10 patients with a histologically demonstrated colon cancer, at a distance always greater than 50 cm from the tumor. Normal mucosal biopsies were provided for comparison from patients who showed no evidence of colorectal disease either on radiological or on colonoscopic examination. Sections of mucosa of normal patients were mounted on the same slides as those from patients diagnosed as having colonic cancer in order to ensure that they were subjected to the same treatment. For each case, 10 slides were immersed into aqueous solutions of (³⁵H)AM (specific activity, 5.2 Ci/mmol; Dickinson and Co., Orangeburg, N. Y.) for 1 hr. They were then washed, dried, and submitted to autoradiography by dipping into an appropriate emulsion (L, Ilford). It should be pointed out that, since the slides were fixed before being exposed to actinomycin, this substance was used not as an antimetabolite but as a "staining" reagent. Background was negligible, and all the grains were located on the nucleus. The nuclear surface of each studied nucleus was measured by means of an inte-

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² The abbreviation used is: (³⁵H)AM, tritiated actinomycin D.
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A grating modular system, composed of a light microscope with a camera lucida. The camera lucida projects a luminous signal onto the microscopic field. Thus, for each cell studied, it is possible to measure both the grain count and the nucleus surface.

Fifty nuclei in the bottom of glands and 50 nuclei in the surface epithelium were analyzed by patients for nuclear surface measurements and grain count. There was a selection of nuclei in the bottom of the glands because of an overlapping of nuclei. Only nuclei in which nuclear size could be measured were chosen in both series.

RESULTS

Normal Subjects. In the colon of normal subjects, hyperplasia was suspected in only 2 cases. The total number of cells, from the bottom to the surface, in longitudinally analyzed half-glands was 49 ± 3.4 (S.D.). Practically all the cells in the lower two-thirds of the crypts were heavily labeled. The mean grain count was 29.1 ± 9.4, and the nuclear surface was 39 ± 14 sq μm. There was no correlation (calculated by linear regression) between grain count and nuclear surface. In the upper third of the crypt, there was an abrupt decrease in grain counts leading to a mean value of 2.9 ± 2 in the most superficial cells. The mean nuclear surface was 38 ± 19 sq μm.

Thus, there is no correlation between grain count and nuclear surface in the crypts of normal subjects. A histogram (Chart 1) of normal colon grain count shows that most of the cells are heavily labeled in the lower two-thirds of the crypts, while very few are labeled in surface epithelial cells (Figs. 1 and 2). The ratio of the grain count at the base of the glands to the grain count in superficial cells is shown for each case in Table 1. All cases show a ratio >4.

Cancer Patients. In biopsies performed in normal-appearing flat mucosa of cancer patients, no true transitional mucosa, as described by Filipe and Branfoot (18) and by Riddell and Levin (27), was observed. The total number of cells in longitudinally analyzed half-glands from the surface to the bottom was the same as in normal patients (46 ± 5 cells). In 2 cases, hyperplasia was suspected, with only a slight increase in the number of cells per glands. The nuclear binding of [3H]AM in these cases was identical to that of the other cases. In all of these 10 biopsies, [3H]AM binding was completely different from that of normal subjects. In the lower two-thirds of the crypts, practically all the cells were heavily labeled. The mean grain count was 27.5 ± 13 with a nuclear surface of 38 ± 9 sq μm. There was a weak correlation coefficient of 0.37 between these 2 values.

On the contrary, in the upper third of the crypts, there is no decrease in [3H]AM binding. The “well-differentiated” surface epithelial cells were heavily labeled with a mean grain count of 22.4 ± 9 but a normal nuclear surface (38 ± 13 sq μm). The correlation coefficient between these 2 values was 0.250. A histogram of grain count (in percentage) shows that almost all of the cells are heavily labeled (Chart 1). The ratio of grain counts in the base of the glands to that in superficial cells is seen in Table 1. The ratio was nearly the same in all cases, and it was always lower than 1.4.

DISCUSSION

The present results confirm our previous work (33), i.e., that in the colon of normal patient the distribution of [3H]AM binding is in correlation with the distribution of proliferating cells (31); the autoradiographic grains are more numerous on the nuclei of the deeper two-thirds of the gland, and they become progressively scarcer as the cells progress to the lumen. In contrast, our present results show that in patients diagnosed as having colonic cancer there is no decrease in [3H]AM binding in the superficial glandular cells, even though they look normal. This larger number of nucleic actinomycin-binding sites (in fixed tissues) can be explained either by the structural organization of the chromatin (24, 25) and the DNA itself (14) or by that of the histones and the acidic proteins (4, 24, 25). Finally, this binding is also restricted by the chromosomal proteins (3,
4, 19, 28). Our results are perhaps related to possible alteration in nuclear nonhistone protein. Indeed, Altrey et al. (1, 6, 7) have demonstrated the presence of 2 acidic proteins (NHP, and NHP2) in human cancer colon, in human adenocarcinoma cultured cells (6), and in experimentally induced rodent colon cancer (7). Besides the fact that these proteins do not exist in the normal human or rodent colon, they appear very early in the cause of carcinogenesis, when no morphological sign of abnormality is apparent (7).

Another possible explanation for our results is the presence, described by Deschner et al. (17) and Maskens and Deschner (23), of well-differentiated epithelial cells labeled by [3H]thymidine at the glandular surface level in normal-appearing flat mucosa of colon cancer patient. These cells could indeed be implicated in the higher level of actinomycin binding that we observed, since it is known from different works (9, 19, 24, 25) that cells engaged in S phase bind [3H]AM more efficiently than do quiescent cells.

Our results demonstrate that normal-appearing colon flat mucosa of the surface of cancer patients contains practically nothing but cycling cells. This higher level of nuclear actinomycin binding does not distinguish between cells in S or G1. It is quite possible that different subpopulations exist. Darzynkiewicz et al. (13) have demonstrated that in neoplasia cells could be blocked at different moments of the cell cycle. The role of nuclear size in cell growth initiation has been demonstrated by Yen et al. (35, 36). In our results, there is a weak correlation coefficient between nuclear size and actinomycin binding in the apparently normal looking flat mucosa of cancerous patients; however, this correlation does not exist in normal individuals. It is of interest to note that the distribution of the ratio (R = ratio of grain count in cells in the bottom of the glands to the grain count in the surface epithelial cells) is much more variable in normal subjects than in cancerous patients (R varies from 4 to 14 in normal patients as shown in Table 1). This can probably be explained by a combination of factors such as variations in genomes, variations in alimentary habits (leading to variable contact with carcinogens), variations in digestive habits (e.g., intensive use of laxatives), and possible hyperplasia. Studies are actually performed in an attempt to answer these questions.

Normal-appearing flat mucosa cells of colorectal cancer patients are probably not involved in a normal process of differentiation. The [3H]AM staining technique of histological sections of fixed biopsies can provide a valuable discriminatory parameter in the screening of early colon cancer.

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REFERENCES

Figs. 1 and 2. [3H]AM binding into epithelial cells of normal-appearing colorectal mucosa of normal subjects (Fig. 1) compared to that of cancer patients (Fig. 2). × 1200.
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