In Vivo Measurements in Familial Polyposis: Kinetics and Location of Proliferating Cells in Colonic Adenomas

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ABSTRACT

In vivo measurements of the proliferation kinetics of epithelial cells in human adenomas were made following i.e. pulse injection of tritiated thymidine ([3H]dThd), in a patient with familial polyposis and Gardner syndrome. In the adenomas and in adjacent flat mucosa, biopsies were taken and microautoradiographs were prepared at intervals after injection. Rates of epithelial cell proliferation, locations of proliferating cells within the crypts of adenomas and adjacent flat mucosa, fractions of [3H]dThd-labeled mitoses and cells, and grain densities were analyzed. Results demonstrated a complete temporal cycle of proliferation of epithelial cells both in adenomas and in flat mucosa, with cells completing only a single cycle during 4 days of observation. Durations of G2 and S phases of the cell cycle were approximately 5 and 15 hr, respectively, both in adenomas and in flat mucosa. After pulse injection, the locations of [3H]dThd-labeled epithelial cells within the adenomatous crypts revealed a large retrograde migration near the lumenal surface; proliferation of labeled epithelial cells in flat mucosa occurred in the lower portion of the colon crypts. The locations of [3H]dThd-labeled epithelial cells at intervals after injection revealed migration rates of 0.4 cell position/hr in crypts of adenomas and 0.3 cell position/hr in crypts of adjacent flat mucosa. However, in contrast to the migration of epithelial cells toward the lumenal surface of the crypts in flat mucosa, in adenomas a markedly abnormal retrograde average migration away from the surface of the mucosa appeared to occur. This retrograde migration was accompanied by an intrusion of the cells into the adenomatous crypt space, contributing to the infolding which accompanies the expanding epithelium inducing the growth of the adenomas.

INTRODUCTION

The possible relationship of benign colon adenomas to colon cancer development has been well described (6, 13). Among major findings have been a correlation between the size of the adenomas and cancer and an increased incidence of carcinoma in adenomas having villi (13). Familial polyposis is a disease of humans characterized by a high frequency of large villous and tubular adenomas of the colon. In this autosomal dominant disease, colonic epithelial cells develop modifications in cell proliferation that precede and accompany the formation of the adenomas (1-5, 10).

Despite the probable importance of adenomas in the formation of carcinomas of the colon, in vivo studies have not been carried out to measure the proliferation kinetics of adenoma-tous epithelial cells. In this study, we have measured several parameters describing cell proliferation in colon adenomas of a subject having familial polyposis; [3H]dThd was injected in vivo to study cell proliferation in this disease. Microautoradiographic measurements revealed (a) a complete temporal cycle of cell proliferation both in adenomas and in flat colonic mucosa, shown by analyzing the rate of appearance of [3H]dThd-labeled mitoses, a corresponding diminution of the intensity of individual cell radioactivity, and the rate of multiplication of the cohort of [3H]dThd-labeled cells; (b) confirmation of previous in vitro studies that show an abnormal concentration of proliferating cells near the luminal surface in the crypts of adenomas; and (c) a retrograde migration of epithelial cells deep into the adenomatous crypts away from the crypt surface, probably contributing to the growth of the adenomas.

MATERIALS AND METHODS

The subject was a 27-year-old woman with familial polyposis who had undergone a resection of most of the colon (partial colectomy) with an ileorectal anastomosis 6 years earlier. The subject had a limited life expectancy due to the development and spread of intraabdominal desmoid neoplasms (i.e., the Gardner syndrome). Five mCi of [3H]dThd (specific activity >25 Ci/mmol; New England Nuclear, Boston, Mass.) were administered as a pulse injection during a 1-min period into the subject's antecubital vein (approved by Clinical Investigation Committee). Biopsies of the rectal mucosa were obtained, using a rigid sigmoidoscope and a proctoscopic biopsy miniforceps, from both adenomas and flat rectal mucosa at convenient intervals during the next 4 days. Two to 4 specimens were taken at the end of each interval and were fixed in neutral formalin-0.9% NaCl solution, embedded in paraffin, and sectioned at 4 μm for microautoradiography with methods described previously (2).

At the end of each time interval, after injection of the [3H]dThd, a number of mitotic cells close to 100 was assayed for labeling in order to determine the fraction of labeled mitoses. Standard 95% confidence limits (15) were assigned to these data. At the end of each time interval, a number of labeled nonmitotic cells in the order of 1000 was examined for grain counts. The number of countable grains found was approximately one order of magnitude larger than this. At the end of each time interval, both for determining the proportion of labeled cells and the crypt height distribution of the labeled cells, several thousand epithelial cells belonging to well-defined crypt columns were examined. Measurements were carried out on individual crypt columns, which were defined as longitudinally sectioned columns of cells that lined the side of a mucosal crypt. Criteria used for counting of labeled and unlabeled cells within a crypt column included the fact that the entire length of the crypt was visible and the crypt reached the muscularis mucosa.

RESULTS

Chart 1A shows the fraction of mitoses found to be labeled in adenomas versus time elapsed after the administration of

1 The abbreviation used is: [3H]dThd, tritiated thymidine.
Chart 1. Fraction of mitoses labeled in adenomas versus time elapsed after administration of $[^3H]dThd$. A, adenomas; B, adjacent flat colonic mucosa. Bars, 95% confidence limits.

$[^3H]dThd$. Chart 1B shows the corresponding labeled-mitosis fraction for adjacent flat colonic mucosa. In both instances, standard upper and lower 95% confidence limits are shown at each measured point by a bar, and the measured points themselves are connected by a smooth curve. Both of these figures show a pronounced initial wave of $[^3H]dThd$-labeled mitoses, as observed previously in gastrointestinal and other rapidly renewing tissues (7, 8, 11, 12) but show little activity following the first wave. In Chart 1A, for the adenomas, a small second wave appears; in Chart 1B, for the flat adjacent mucosa, there is also a suggestion of a second wave of very low amplitude. However, the general appearance in both cases reveals a near cessation of cell proliferation following the initial wave of mitoses. In both instances, the lack of a large-amplitude wave of mitosis following the first wave probably indicates the removal of cells from active proliferation; dispersion of cell cycle rates could also contribute to the findings.

With just a single distinct wave of labeled mitoses present, only some of the conventional cell cycle time parameters (14) can be determined with any degree of assurance from the data. The durations of the G2 and the S phases are observed to be in the order of 5 hr and 15 hr, respectively, both in the adenomas and in the adjacent flat mucosa. Estimates of the duration of G1 phase and of the total cell cycle time would not have good precision if made from the present data and are not included. The durations observed for G2 and S phases are similar in magnitude to those reported previously for normal colonic epithelial cells following in vivo administration of $[^3H]dThd$ to humans (3, 7–9, 11, 12); more variable measurements have been observed in colon carcinomas (3, 9).

Chart 2 shows $[^3H]dThd$-labeled the adenomas and for the flat mucosa, respectively. These data show a decrease to roughly one-half of the initial value in a period that corresponds to the duration of the initial wave of labeled mitoses in Chart 1, and no further decreases thereafter. Comparison of the number of grains per labeled cell immediately after injection with those observed after the initial wave of labeled mitoses using the 2-sample t test revealed significant differences (p < 0.01 in each instance). During this period, the percentages of colonic crypt cells that were found to be labeled approximately doubled, reaching 20% for the adenomatous crypts and 7% for the flat mucosal crypts. These findings indicate a dilution per cell of the initial $[^3H]dThd$ labeling by a factor of 2:1 over the time period observed, indicating that a single cell division had occurred on the average. The results are consistent with a cessation of cell proliferation following the first wave of mitoses, as noted from Chart 1.

Chart 3 shows the measured distribution of labeled cells within adenomatous crypts at different times after $[^3H]dThd$ labeling. Chart 4 shows the corresponding distribution of labeled cells within crypts in the adjacent flat mucosa. These distributions of labeled cells have certain regularities. All of the labeled cell distributions for flat mucosa (Chart 4) are peaked near the base of the crypt as observed previously in normal mucosa (1–5, 8, 10–12), and the corresponding distributions for adenomas (Chart 3) are all peaked closer to the lumenal surface of the crypt. In both adenomas and flat mucosa, the mean position of the labeled cells can be fitted by linear regression versus time. There are opposite slopes, however, for the adenomas and the flat mucosa, as shown in Chart 5.

Chart 5 gives the mean position of labeled cells versus time of assay for adenomas and flat mucosa. Chart 5B shows a clear trend (correlation coefficient, 0.95) for the mean position of labeled cells in flat mucosa to migrate upward in the crypt, away from the crypt base. This is in accord with previous observations of normal repopulation of the mucosal epithelial cell lining of colonic crypts (3, 7, 9, 11, 12). The mean rate of upward migration is approximately 0.3 cell positions/hr, with an initial intercept at Cell Position 21 above the base of the crypt.
Chart 3. Distributions of labeled cells within adenomatous crypts at different times after [3H]dThd labeling. Individual distributions (A to F) correspond to intervals from 12 to 86 hr shown in Chart 5A. Cell position zero is at the luminal surface of the crypt, and position numbers increase away from the surface and deeper into the crypt.

Chart 4. Distributions of labeled cells within crypts of flat mucosa at different times after [3H]dThd labeling. Distributions (A to G) correspond to intervals from 4 to 86 hr shown in Chart 5B. Cell position zero is at the base of the crypt, and position numbers increase towards the luminal surface of the crypt.

Chart 5. Mean position of labeled cells versus time of assay for adenomas and flat mucosa.

Crypt. Chart 5A, in marked contrast, shows a slightly less well-defined trend (correlation coefficient, 0.81) for the mean position of the labeled cells in adenomas to diffuse downward in the crypt, away from the luminal surface. Here, the rate of downward motion of the mean position is approximately 0.4 cell position/hr, with an initial intercept at Cell Position 32 counting downward from the luminal surface. This retrograde average migration of labeled cells in adenomas, which is evident from the data in Chart 5A, has not been observed previously, although clustering of labeled cells close to the luminal surface in adenomas has been noted in vitro (1-5, 7, 9, 10). In the data recorded in Chart 5, both slopes were significantly different from zero using the t test for simple linear regression (p = 0.05 and p < 0.01, respectively).

DISCUSSION

In vivo administration of [3H]dThd to a human subject having symptomatic familial polyposis has permitted analysis of several aspects of cell proliferation kinetics in colonic adenomas and comparison with the corresponding kinetics for adjacent flat mucosa. Both in flat and adenomatous tissue, emphasis was placed on measuring the proportion of mitoses that were labeled and on determining the locations of labeled epithelial cells in assayed crypts versus time following administration of [3H]dThd.

During the half-week period of observation, the time dependence of the fraction of mitoses labeled and of grain counts over labeled cells indicated that in both adenomatous and flat mucosal crypts cells went through essentially only a single proliferative cycle with a doubling of the number of cells. These
observations, which include the finding of a well-defined cycle of cell proliferation and estimation of the durations of its G₂ and S phases, are the first in vivo observations of colonic adenomas. The time behavior of proliferating cells in adenomatous crypts was similar to that observed in neighboring crypts of flat colonic mucosa of the same subject, and this in turn was similar to that of colonic crypts in normal subjects.

The present study confirms, for the first time in vivo, information known previously from in vitro studies (1–5, 10) that in crypts of colonic adenomas active proliferation of epithelial cells anomalously occurs close to the luminal surface (Chart 3). For the first time, it was determined that in flat mucosa in familial polyposis an upward migration of proliferating epithelial cells occurs, away from the base of the crypt (Chart 5B), similar to what is observed in the colonic crypts of normal subjects. In adenomatous crypts, however, there appears to be an average migration of newly formed epithelial cells downward in the crypt, away from the locations near the luminal surface where abnormal proliferation occurs (Chart 5A).

The anomalous downward migration of epithelial cells in adenomatous crypts is about as rapid as the normal upward migration of epithelial cells in flat mucosal surfaces. This retrograde cell kinetic behavior of the adenomatous crypts, which have developed an ability to accumulate and to be retained in the adenomatous mucosa, is further suggestive of the disordered cell kinetics of malignant colonic epithelial cells; it tends to support the current concept of progressive multiple stages in these cells leading to cancer. Although the epithelial cells in colonic adenomas do not invade the submucosa, which would be a criterion of malignancy, their downward average motion in the adenomatous crypts does constitute a continuous intrusion upon the colonic crypts space by its own epithelial cells. This rate of intrusion appears to be quite large. From the regression line in Chart 5B, it is close to 0.4 cell position/hr or 3500 cell positions/year. This latter number of cell positions is large compared to the entire length of a crypt, even in large adenomas where crypt lengths as long as a few hundred cells can occur.

If the anomalous downward migration of adenomatous epithelial cells leads them to accumulate as part of an expanding crypt epithelium, it would provide a mechanism for the growth of the adenomas. On the basis of this concept and the observed average epithelial cell intrusion rate of 0.4 cell position/hr, estimates can be made of the rates of growth of adenomas. The estimates at rates of adenomatisional factor contributes to the growth rate. Thus, some of the intruding epithelial cells, perhaps as many as one-half, may slough off internally to the crypt and be excreted through the mouth of the crypt. Alternatively, growth rates of adenomas are known to vary markedly in familial polyposis, at times even in the same individual. Studies can be devised to further investigate the factors contributing to the retrograde migration of adenomatous epithelial cells and the further progression of these cells to malignancy.

REFERENCES

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