Early Proliferative Changes in Intestinal Cells

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Summary

Early lesions in the colonic mucosa of humans and rodents are characterized by similar proliferative changes within their epithelial cell population. Progressive phases of abnormal cell development appear during the evolution of neoplastic transformation in colonic cells of rodents exposed to chemical carcinogens and in humans highly susceptible to gastrointestinal cancer. Identification and classification by phenotype of cells of these individuals at increased risk for colon cancer are leading to new methods to improve the detection and diagnosis of neoplasia in high risk individuals and families. An analytical system of precise numerical definitions is aiding an approach to modify the evolution of advanced stages of neoplasia.

Introduction

In this session on early lesions in gastrointestinal cells, papers will be presented that describe abnormal morphological and biochemical events developing in both humans and rodents.

This paper will describe recent studies on the evolution of neoplastic transformation in the cells of individuals highly susceptible to gastrointestinal cancer and similarities in abnormal proliferative activity occurring in rodents exposed to chemical carcinogens. A quantitative analysis will be described which has been used to study the evolution of the stages of cell transformation in humans (11). These observations of early lesions are leading to new means of detection and diagnosis of neoplasia in high risk individuals and families. An analytical system of precise numerical definitions is aiding an approach to modify the evolution of advanced stages of neoplasia.

Abnormal Proliferation of Colonic Epithelial Cells during the Stages of Neoplastic Transformation in Inherited ACR

The dominant inherited disease ACR (familial polyposis) shows a clear illustration of the abnormal phases of cell growth in individuals who are highly susceptible to the development of colon cancer. The sequence of abnormal cell growth and neoplastic lesion formation is manifest when colonic epithelial cells develop an increased ability to proliferate and accumulate in the mucosa (4, 11). Normal cells undergo a rapid differentiation during their migration to the surface of the colon. Concomitant with their migration there is a repression of DNA synthesis and proliferative activity.

A failure of repression of DNA synthesis in the disease state represents the earliest phenotypic expression identifying a genotypic proclivity for cell transformation (Chart 1). This characteristic, however, is not an exclusive indication of neoplasia, for it occurs in cells before they develop additional required characteristics and before they begin to
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Chart 1. A, location of proliferating and differentiating epithelial cells in normal colonic crypt. Dark cells illustrate thymidine labeling in cells that are synthesizing DNA and preparing to undergo cell division. B, colonic cells that fail to repress the incorporation of thymidine into DNA and begin to develop an enhanced ability to proliferate.

Table 1
Colonic mucosa of patients with inherited ACR (familial polyposis)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Mucosa adjacent to polyp</th>
<th>Polyp</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, m</td>
<td>F</td>
<td>59</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2, d</td>
<td>F</td>
<td>24</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>34</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4, f</td>
<td>F</td>
<td>7</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5, d</td>
<td>F</td>
<td>35</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6, a</td>
<td>M</td>
<td>17</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7, n</td>
<td>F</td>
<td>26</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8, s</td>
<td>M</td>
<td>33</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9, b</td>
<td>F</td>
<td>32</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>10, m</td>
<td>M</td>
<td>17</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11, son</td>
<td>F</td>
<td>41</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>12, b</td>
<td>F</td>
<td>20</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>13, b</td>
<td>M</td>
<td>37</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>9/14</td>
<td>14/14</td>
<td></td>
</tr>
</tbody>
</table>

a Siblings (from Ref. 4).
b Brace denotes family.

accumulate as polyps. An accurate identification of neoplastic transformation includes other observable cellular changes during lesion evolution. Table 1 illustrates the frequent occurrence of a failure of cells to repress DNA synthesis during migration, in mucosa adjacent to polyps in patients with inherited ACR. In Table 2 it is seen that relatives of these patients, who had been considered clinically negative because they had not developed gross polyps, also have colonic epithelial cells that fail to repress DNA synthesis. The finding was observed in isolated patchy segments rather than throughout the colonic mucosa and occurred with higher frequency in these individuals compared to previously observed control groups.

Chart 2 illustrates a 2nd observable phenotypic change in the inherited disease, occurring in those cells that are no longer capable of repressing DNA synthesis. Its expression is defined by the accumulation of cells in the colonic mucosa and their subsequent initiation of polyp formation. In individuals with inherited ACR, abnormal accumulations of cells labeled with tritiated thymidine are found near the surfaces of colonic adenomatous polyps. In these lesions there is an elevation of the activity of thymidine kinase, an enzyme catalyzing the phosphorylation of thymidine prior to its incorporation into DNA (17).

Table 2
Rectal mucosa of relatives of familial polyposis patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>[3H]Thymidine labeling of cells at surface and upper one-fifth of crypts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>48</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>43</td>
<td>+</td>
</tr>
<tr>
<td>3 b</td>
<td>F</td>
<td>45</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
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<td>-</td>
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<td>5</td>
<td>F</td>
<td>25</td>
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<td>6</td>
<td>M</td>
<td>25</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>14</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>6/7</td>
<td></td>
</tr>
</tbody>
</table>

a Siblings (from Ref. 4).
b Brace denotes family.
An additional characteristic of transformed cells also has recently been found in inherited adenomatous of the colon and rectum. It is expressed by the ability of normal-appearing skin fibroblasts of affected individuals to grow in medium containing low serum when plated at low density (14), more efficiently than normal cells, a finding that may be associated with the genetic defect leading to abnormal growth of colonic cells.

Effects of Chemical Carcinogens in Neoplastic Transformation of Colonic Epithelial Cells

The chemical carcinogens DMH (2, 8, 12, 15, 16, 19), methylazoxymethanol (20), and N-methyl-N'-nitro-N-nitroso-guanidine (10, 18) have been used to induce morphological and proliferative changes in the colonic epithelial cells of rodents. Investigators have described variations in carcinogenic sensitivity to colon cancer among different rodent strains (7, 9).

The observations that follow are of interest, as these aspects of rodent induced carcinogenesis show similarities to neoplastic transformation observed in human cells. For example, mice exposed to DMH show more cell proliferation in the induced adenomas than in the adjacent flat mucosa (2, 5). This has also been observed in inherited ACR in humans.

In both DMH-induced carcinomas of mice, and in humans, the distal colon has been the major region of tumor nodule distribution. Observations in the mouse showed that multifocal tumors (e.g., adenomatous polyps, carcinomas, metaplasias) protruded into the lumen of the rectosigmoid following abnormal proliferation and accumulation of cells in mucosa. Progressive pathological changes in mice and rat range from early focal atypias and hyperplasias (mainly on the mucosal folds) to adenomatous polyps and carcinomas. Increased cellular proliferative activity has been observed to accompany these changes. Of particular interest in comparison to humans is the fact that in some areas of flat mucosa the proliferative zone extended toward the surface of the colonic crypts, after DMH (16) and N-methyl-N'-nitro-N-nitroso-guanidine (10).

Other features of carcinogenic tumor induction in mice have also been observed. There is a shift of proliferating epithelial cells into expanding adenomas and carcinomas. Cells have shown continued DNA synthesis throughout most of their life-span, with an increase in both the total number of thymidine-labeled cells and their position up from the crypt base (16, 19). Rodent DMH-induced tumors expanded into the colonic lumen with a proliferation of neoplastic epithelial cells near the lesion surface. Table 3 summarizes these observations.

The similar findings in humans with increased susceptibility to colon cancer, and in rodents after carcinogen treatment, support the possibility that these are common steps leading to the neoplastic transformation of colonic cells with a common defect developing in the regulatory control of cell proliferation. The role that chemical carcinogens may have in the neoplastic transformation of colonic epithelial cells of humans is also supported by these findings.

Utilizing the Stages of Preneoplasia in Predicting the Evolution of Neoplastic Lesions of the Colon

Identifying the phases of cell transformation is useful for the development of predictive indices related to the evolution of colonic neoplasms. We have begun to quantitate the appearance of these changes in individuals at varying degrees of risk of colon cancer as they occur individually and simultaneously. In populations at increased risk of cancer the fractions of cases having simultaneous preneoplastic changes have been identified. The degree of association between 2 phenotypic traits is quantitated in terms of 2 ratios. The 1st ratio identifies the fraction of all individuals displaying 1 of the traits that occurs in the subpopulation containing the remaining trait. The 2nd ratio identifies the corresponding fraction in which the order of the 2 traits is reversed. This information bears on the prediction of future disease and on interrelationships of the phenotypic traits that are of etiological interest.

An example of the utility of the analysis is seen in Table 4 where relationships between the phenotype s' (a failure of colonic epithelial cells to repress DNA synthesis) and n' (an ability of s' cells to accumulate in the mucosa, initiating neoplastic lesions) are shown. In our current high-risk population group n' leads to colon cancer with a probability of near 1.

In Table 4 our current observations in the high-risk population group with inherited ACR indicate that, in the Venn diagrams generated by the analysis, the fraction of cases having s' in flat colon mucosa is 0.85, the fraction having n' is 0.64, and the fraction simultaneously having s' detected in flat mucosa and also developing n' is 0.49. Modification of these values will occur as new cases accumulate. The current analysis also indicates that the development of s' may be etiologically related to the development in n', for s' is present in all adenomatous polyps.

This approach offers a basis for the identification and quantitation of new abnormal phases of cell development
leading to transformation in human population groups. It can provide a statistical definition for predicting the evolution of new and more advanced stages of neoplasia.

It is anticipated that this type of information will describe more accurately the expression of abnormal phenotypic traits and thus improve the surveillance and treatment of high-risk individuals and families. Furthermore, the development of this analytical system of precise numerical definitions has now offered our laboratory an opportunity to attempt to modify the evolution of more advanced stages of neoplasia with preventive measures in humans.

References
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