Cell Differentiation and the Development of Colonic Neoplasms

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This year about 75,000 Americans will develop cancer of the colon and rectum. These lesions are now responsible for a greater number of cancer deaths than any other single organ site with the exception of lung. Annual mortality rates of colon and rectal cancer are higher in the United States than in many other countries with differences partly due to different age distributions of the populations. However, similar ethnic groups living in geographic areas adjacent to one another also develop differences in frequency and immigrant groups coming to the United States tend to develop the incidence rate of our country. Thus, evidence is available to indicate that environmental factors have a role in the development of these lesions.

Additional data support the view that constitutional and hereditary factors also have a role. For example, in a number of syndromes, hereditary transmission of colonic polyps and carcinomas has been observed. In familial polyposis, a multiplicity of carcinomas of the colon develops with early age of onset, indicating that the colon is fertile ground for the development of cancer.

In nonfamilial disease, the antecedent mucosa that gives rise to adenomatous polyps and villous papillomas also has been implicated in the development of colonic cancer. When patients with one colonic cancer have additional adenomatous polyps, they have a greater chance of
developing second carcinomas. Similarly, the risk of developing a second colon cancer is increased in patients who previously had a colon carcinoma.  

What are some of the changes that take place within the epithelial cells of the colonic mucosa and within these lesions before carcinomas appear? Recent studies have demonstrated an increasing number of abnormalities of nucleic acid metabolism. During the development of these lesions, colonic epithelial cells of man develop an increased capacity to proliferate. Simultaneously, the normal differentiation of the cells is impaired.

THE NORMAL COLONIC CELL

When contrasted with normal cells, many differences are observed. The milieu that gives rise to the development of normal colonic cells is one in which rapid change is the rule. As the new cells migrate from the deeper regions of the mucosa to the surface of the crypts, the structure and function of the cells change rapidly. Well known developments include increases in endoplasmic reticulum and membrane-bound particles of ribonucleoprotein, formation of microvilli, and increase in enzymes such as alkaline phosphatase.  

Deoxyribonucleic acid (DNA) synthesis also comes to a halt. Metabolic activities having a role in DNA synthesis and cell proliferation normally decrease, as is seen in the decrease in activity of the enzyme thymidine (TdR) kinase during normal cell migration and differentiation. This decrease has been observed in small intestine and colon. In the synthesis of DNA, thymidylate synthetase serves as a major enzyme to catalyze the conversion of deoxuridine monophosphate (dUMP) to thymidine monophosphate (dTMP). The latter can also be formed from TdR by the enzyme TdR kinase. TdR kinase catalyzes the phosphorylation of TdR to the monophosphate derivative, which then is converted to the triphosphate that becomes part of the DNA molecule. The enzyme is present in a wide variety of cell types and is found where cells are making DNA and are about to divide. During migration of epithelial cells of the small intestine and colon, activity of this enzyme disappears as cell proliferation stops and the cells develop the morphological and biochemical characteristics of mature, well differentiated cells. It is not known whether transcription of the enzyme stops, or whether a conformational change in its molecular structure might be induced to account for its inactivation. Whatever the reason for its disappearance, the on and off activity of this enzyme correlates very well with the reproduction of normal small intestinal and colonic epithelial cells.

The development of other nucleic acid enzyme activities also accompanies the
differentiation of normal small intestinal and colonic epithelial cells as they migrate to the surface area of mucosa occupied normally only by nonproliferating cells. Enzymes catalyzing metabolic reactions that lead to the synthesis of nucleotide precursors of RNA increase in activity. Purine nucleoside phosphorylase, AMP: and IMP: pyrophosphate phosphoribosyl transferases, in addition to adenosine deaminase, increase markedly during normal cell differentiation and migration. Among their functions, the transferase enzymes probably facilitate the reutilization of nucleic acid precursors from extruded cells, thus decreasing the body's requirement for de novo biosynthesis of nucleotides.

Different regulatory controls appear to be responsible for bringing about the development of these new metabolic pathways and for raising and lowering the levels of enzymes that are active in nucleic acid metabolism as the cells undergo differentiation. Recent experiments with cycloheximide and actinomycin D (which indicate comparative rates of synthesis and degradation of these enzymes and their templates) have shown differences in the life span of these important constituents of migrating intestinal cells. TdR kinase had a very short half-life. Adenosine deaminase was rapidly synthesized and degraded, implying continual use of the genetic material from which it was produced and rapid and sensitive control of the enzyme level during the stages of cell differentiation. On the other hand, purine nucleoside phosphorylase and its template had a long life span and were more stable in the cells, being less sensitive to control at the gene level. Differences in the rate of replacement of these enzymes and their templates and in their stability have given some insight into the fact that a multiplicity of operational controls are built into normal intestinal cells to bring about the appearance and disappearance of metabolic pathways during critical stages of cell differentiation.

During the embryonic development of cell types throughout the body, the cells progressively lose their ability to express differing patterns of metabolic activity that might transform them into other cell types. In an analogous manner, as normal adult intestinal cells undergo differentiation, metabolic pathways connected with the proliferative process decrease. For many years it was suspected that the regulatory controls governing the cessation of proliferative activity and the development of new functions in differentiating cells were influenced by both nuclear and cytoplasmic factors. Studies on limb regeneration in amphibian species and studies on the transplantation of embryonic nuclei into other cells contributed to this concept. Other data showed that much of the genome is inactive normally and specialized ribonucleic acids are synthesized in different cell types of higher species. Recently, Gurdon has provided evidence that cytoplasmic factors have a role in the regulation of gene activity during cell differentiation. By transferring intestinal cell nuclei of tadpole to eggs whose nuclei were not functioning, he showed that genetic information contained within the transferred nucleus could be derepressed so that an entire organism developed. These lines of evidence have indicated that each state of differentiation is normally influenced by a set of genes active in transcription, while other genes remain inactive, repressed by cytoplasmic factors that act in concert with the genome or other elements in the nucleus.

THE ABNORMALLY PROLIFERATING COLONIC CELL

In well differentiated surface epithelial cells in the colon of man that still appear histologically normal, an early abnormality that develops and that persists as polypoid lesions form is continued DNA synthesis. Whether derepression at the level of the genome initially occurs in these well differentiated cells is not known. It is possible that critical regulatory controls
leading to the cessation of DNA synthesis never develop during the life of the cells. An initial approach to an analysis of these possibilities has involved the study of nucleic acid metabolic pathways in the abnormal cells of colonic mucosa and the polypoid lesions that develop there.

In areas of colonic epithelium containing cells of this type, the anatomical boundary or transitional zone that normally separates proliferative cells from nonproliferative mature surface cells is lost. Migration through this zone in the midportion of the colonic crypts, which normally is accompanied by activation of the kinds of regulatory controls described above to yield well differentiated nonproliferative cells, no longer produces that kind of cell. Instead, some cells move through the zone while they retain the capability to divide. These cells are always found with hyperplasia of the mucosa and may be found without it. Here, the normal steady state proliferation kinetics of the cells have changed and increased numbers of cells accumulate in the mucosa. These cells also make greater amounts of RNA and protein than normal mature colonic cells and in these parameters as well as in continued DNA synthesis they function as immature colonic cells making new protoplasm for daughter cells. As these cells migrate through the mucosa, their ability to escape the inhibition of mitosis that accompanies normal cell differentiation can be viewed as a success for the individual cell in terms of reproduction but a failure in the development of a normal mucosa. These cells are able to propagate their species in new locations where formerly they could not. Colonic mucosa, that gives rise to adenomatous polyps and villous papillomas, and the surfaces of the lesions contain cells of this kind. With accumulation of increasing numbers of these cells, the lesions increase in size. In villous papillomas junctions between cells also become scant. Measurements of nucleic acid enzyme activity in the cells of these lesions confirm the fact that these cells are not well differentiated biochemically. In the surface cells of some areas of colonic mucosa near polyps, TdR kinase activity is high. TdR kinase activities in the surface cells of villous papillomas and carcinomas also are high, similar to the activity seen in young proliferative cells rather than mature cells. Other enzyme activities that characterize cell differentiation do not appear in the polypoid lesions. AMP: and IMP: pyrophosphate phosphoribosyl transferase activities fail to develop. These enzymes decrease progressively in mature colonic cells, adenomatous polyps, villous papillomas, and carcinomas. Comparative activities of these enzymes decrease as the lesions increase in malignant potential and these important metabolic pathways of mature functioning cells do not develop normally.

Chromosome abnormalities also have been observed in these lesions in several studies, with hyperplody in villous papillomas and adenocarcinomas. Trisomy has been observed in the cells of adenomatous polyps, with the additional chromosome not from the same group. A suggestion of linkage between the genetic transmission of familial polyposis and presence of Duffy blood group also has been made, but the data are not conclusive.

Many other related abnormalities undoubtedly could be detected in the cells of polypoid lesions and the mucosa that gives rise to these lesions. Many of the regulatory controls that lead to the expression of mature cell activity and cessation of proliferation have not been analyzed, nor have ectopic molecular structures or abnormal properties of essential cell constituents that might be present. Environmental carcinogens also may be active in these lesions, but none has been identified in humans. In colonic carcinomas of man, however, several abnormal molecules have been identified recently. A carcinoembryonic antigen and an isoenzyme of alkaline
phosphatase have been detected. In addition to giving further insight into the pathogenesis of carcinoma, routine identification of these abnormal products of the colonic cancer cell could possibly lead to clinical tests useful in the detection of the disease after it has developed. It may be of equal interest to determine whether biochemical and immunological measurements can indicate the future malignant potential of colonic lesions before they have developed into full-blown cancers. To be able to describe routinely, in broader terms than is currently given by morbid pathology, the stage of development of these benign and malignant lesions would be a significant step forward. The multiplicity of developmental errors now becoming apparent in colonic epithelial cells prior to and during the appearance of malignancy indicates this possibility.

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