Journal of Cellular Biochemistry, Supplement 16G:1-13 (1992)

Gastrointestinal Cancer: Pathogenesis, Risk Factors and the Development of Intermediate Biomarkers for Chemoprevention Studies

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Abstract Dietary, environmental and genetic factors contribute to the etiology, pathogenesis and risk for gastrointestinal cancers. Measurements of cell proliferation and differentiation further identify abnormal cellular properties associated with increased susceptibility to gastrointestinal cancer. In precancerous esophagus, the proliferative compartment increases in size, increased ploidy and dysplasia develop, and epithelial cells express abnormal cytokeratins and ectopic tumor-associated antigens. In precancerous stomach, increased proliferative activity and metaplasia develop. Intestinal enzymes and mucins are expressed and normal gastric antigens are replaced by intestinal or embryonic antigens. In flat colonic mucosa and in colonic adenomas, expansions of the proliferative compartment occur. Gene expression is modified, gene deletions occur and blood group-related antigens are modified as the cells undergo abnormal differentiation and develop into adenomas and carcinomas. Chemopreventive regimens are now being tested to determine whether they modify such intermediate biomarkers toward normal levels characteristic of lower risk for neoplasia. It is anticipated that the utilization of intermediate biomarkers in chemoprevention studies may permit more novel chemopreventive regimens to be tested in human subjects than heretofore was possible.

Key words: biomarkers, chemoprevention, differentiation, gastrointestinal cell proliferation, intermediate biomarker

INTRODUCTION

In earlier and more recent studies, an ability of investigators to measure parameters related to cell proliferation and differentiation have revealed changes in gastrointestinal epithelial cells occurring in individuals with increased susceptibility to neoplasia. During the development of precancerous diseases in humans and after exposure of rodents to carcinogens, DNA synthesis continues in the cells as they migrate to the surface of the epithelial lining where the cells do not differentiate normally. Further changes occur as precancerous diseases become more advanced and gastrointestinal cells exhibit progressively increasing degrees of abnormally delayed maturation.

These findings develop both in humans who have diseases predisposing to cancer, and in rodents after treatment with chemical carcinogens: as the precancerous diseases develop, abnormally proliferating cells eventually accumulate in the epithelial lining. This occurs in all cancer-prone regions of the gastrointestinal tract, i.e., in esophagus, stomach and colon [1].

It is now possible to identify proliferating cells in tissue sections of gastrointestinal epithelium by measurements that (1) incorporate tritiated thymidine ([^{3}H]dThd) into newly synthesized DNA of cells in S phase of the proliferative cell cycle, identified by microautoradiography; (2) incorporate BrdU into newly synthesized DNA of S phase cells, identifying the cells with antibody to BrdU using immunoperoxidase assay; (3) identify proliferating cell nuclear antigen (PCNA) in cells in S and other phases of the cell cycle, with antibody to PCNA and immunoperoxidase assay; and (4) identify cells that are

doubly labeled both with [^{3}H]dThd and PCNA, facilitating the identification of multiple phases of the proliferative cell cycle including S, G_1, and G_0. Figure 1 illustrates the identification of proliferative cells in the gastrointestinal tract by application of these methods.

DISEASES OF THE ESOPHAGUS

In studies that have used the incorporation of [^{3}H]dThd into proliferating cells, the size of the proliferative compartment has been shown to expand in several esophageal diseases that lead to increased frequencies of cancer (Table 1). As dysplasia occurs increased numbers of proliferating epithelial cells also accumulate in the esophageal basal layer. Barrett's disease of the esophagus leads to a marked increase in the incidence of esophageal cancer, and may account for a large fraction of the esophageal cancer occurring in the United States [2]. In this disease immature epithelial cells accumulate in the esophageal lining and some undergo metaplastic changes; proliferative cells sometimes reach the surface of the esophageal lining as the proliferative compartment expands [3].

In Linxian, China, a high incidence of esophageal cancer has been found to occur. Cumulative death rates from esophageal cancer have been reported to be as high as 33% for males and 20% for females [4]. In this high-risk region of China and in other geographic regions of high esophageal cancer incidence, squamous epithelial cells gradually undergo morphological changes from normal to hyperplasia to dysplasia before the onset of cancer. In precancerous esophageal disease in Linxian, as hyperplasia and dysplasia developed, the proliferative compartment progressively increased in size, and the numbers of proliferative cells markedly increased [5] (Figure 2).

As normal esophageal squamous epithelial cells differentiated they expressed other biomarkers (Table 2) including different molecular species of keratins [6] similar to squamous epidermal cells. However, when esophageal epithelial cells became malignant they expressed cytokeratins not present in normal esophageal cells [7]. Esophageal carcinoma cells also developed various ectopic tumor-associated antigens [8].

DISEASES OF THE STOMACH

In diseases of the stomach that led to increased frequencies of human gastric cancer, characteristic changes occurred in the proliferation and differentiation of epithelial cells. The development of gastric carcinoma was preceded by increased proliferative activity of gastric epithelial cells (Table 1), and by intermediate stages of abnormal cell differentiation, including metaplasia of small and large intestinal types and dysplasia (Table 3). In these diseases, proliferating epithelial cells failed to differentiate normally as they migrated to the surface of the mucosa, and immature cells lined the gastric surface, directly contacting the contents of the stomach.

Thus, in chronic atrophic gastritis, a hyperproliferation of gastric epithelial cells developed and increased numbers of cells replicated more rapidly than normal, and migrated more rapidly than normal to the surface of the epithelial lining where immature epithelial cells were extruded from the surface. In gastric atrophy, the immature proliferative epithelial cell compartment also expanded towards the surface of the gastric mucosa. (e.g., 9).

Recent findings in peptic ulcer disease [10] have indicated that gastric epithelial cell proliferation rates were similar to mild gastritis without atrophy and minimal gastric atrophy. However, cell proliferation progressively increases with increasing gastric atrophy,

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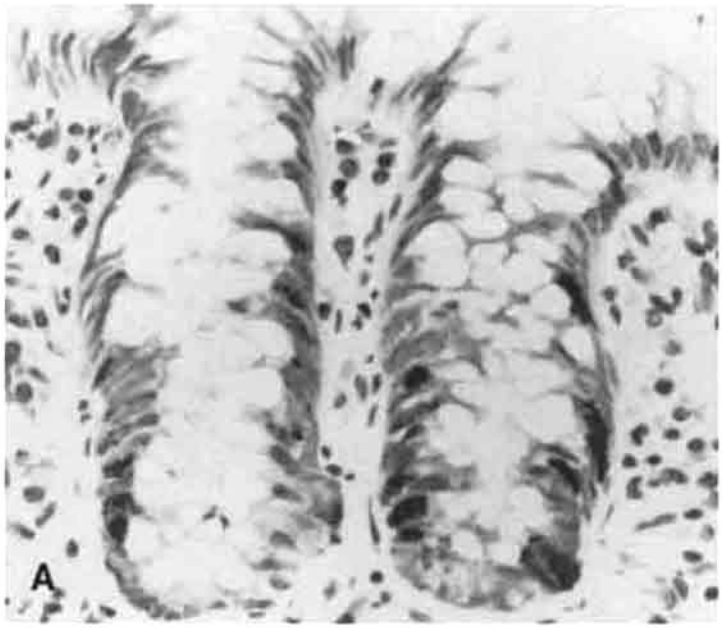
Fig. 1A. Example of [^{3}H]dThd incorporated into newly synthesized DNA of human colonic crypt epithelial cells in S phase of the proliferative cycle, identified by microautoradiography. **Fig. 1B.** Example of bromodeoxyuridine (BrdU) incorporated into newly synthesized DNA of rat colonic crypt epithelial cells in S phase of the proliferative cycle, identified with immunoperoxidase assay using monoclonal antibody to BrdU. **Fig. 1C.** Example of proliferating cell nuclear antigen (PCNA) in colonic epithelial cells of rat colon identified with immunoperoxidase assay using monoclonal antibody to PCNA. **Fig. 1D.** Double labeling of cells both with [^{3}H]dThd and PCNA, cells labeled with PCNA alone, and cells unlabeled by [^{3}H]dThd or PCNA, facilitating identification of multiple phases of the proliferative cell cycle (measurements by Dr. K. Yang).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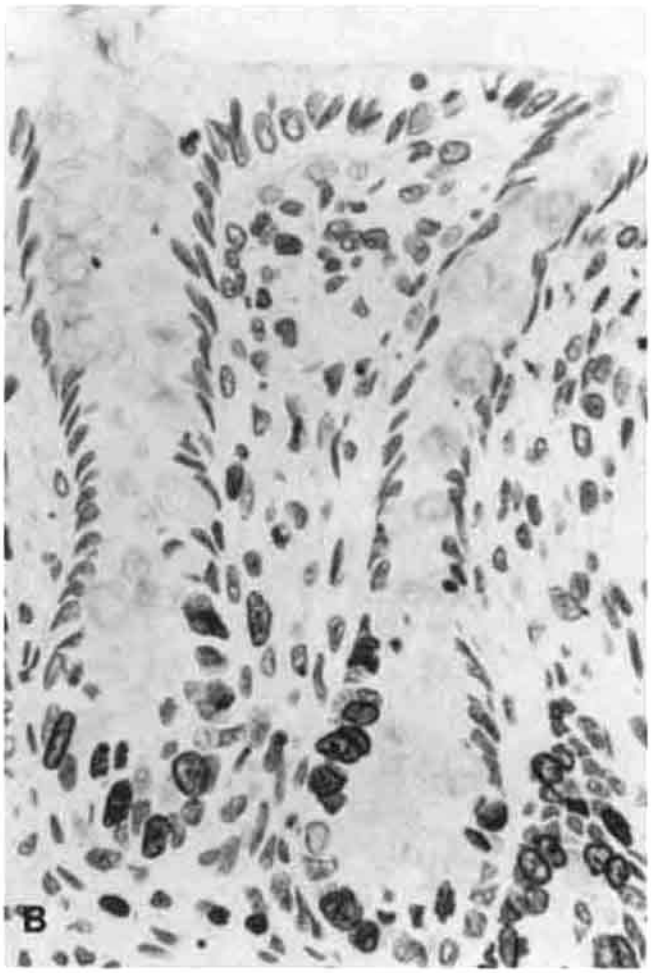
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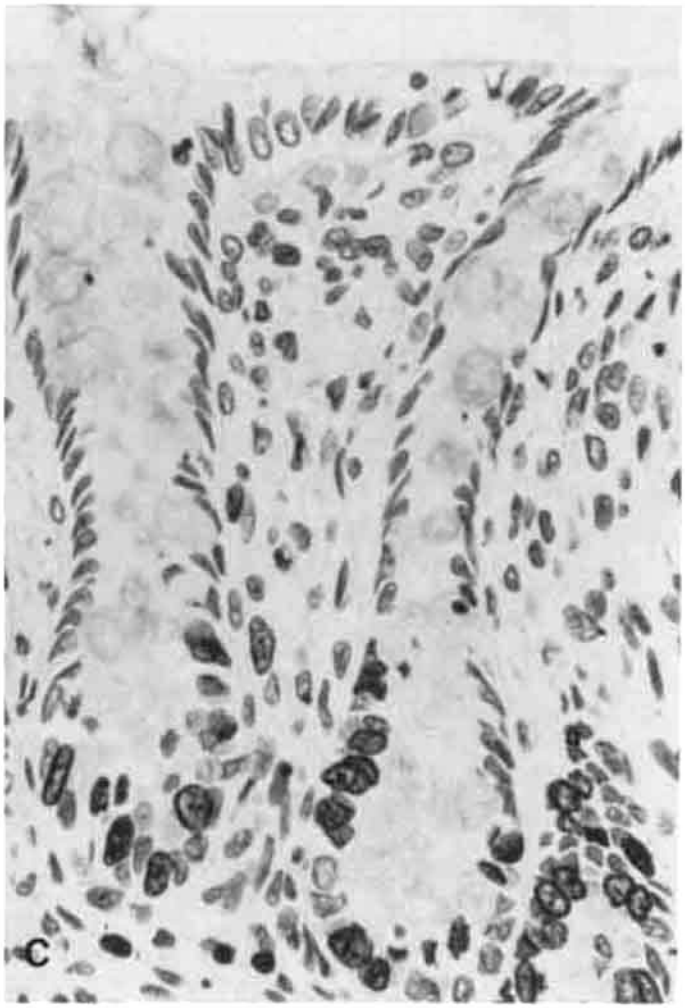
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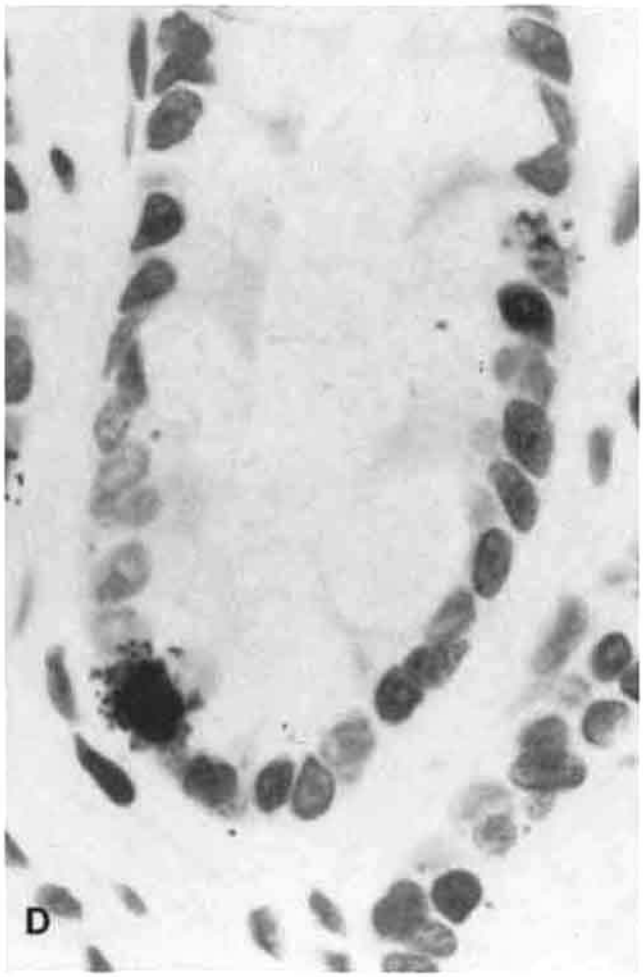
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TABLE I. Expansion of the Proliferative Compartment of Epithelial Cells: Studies of Human Subjects

Organ	Diseases	References
Esophagus	Barrett's esophagus	3 29
	Esophagitis, reflux	30
	Esophagitis, Linxian	31 5
Stomach	Pernicious anemia	33 34
	Chronic gastritis	35 9 10 11 36
	Partial gastrectomy	37 38 12

TABLE II. Biomarkers of Abnormal Differentiation of Gastrointestinal Epithelial Cells: Esophagus

Organ	Biomarker	References
Esophagus	Histopathology of hyperplasia and dysplasia	39 5
	Ectopic and normal cytokeratins	6 40 41 7
	Tumor associated antigens	8
	Modified response to growth factors	42

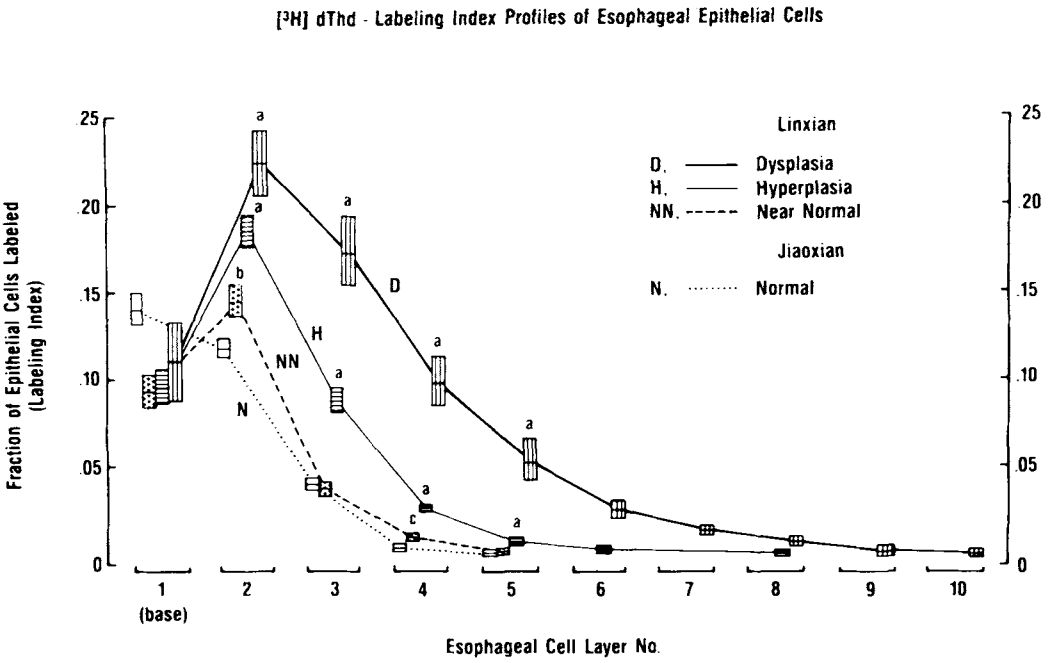

Fig. 2. Expansion of the proliferative compartment of human esophageal cells in biopsy specimens of hyperplasia and dysplasia of the esophagus (from Reference 5).

reaching a peak in severe atrophy and gastritis as cells that do not terminally differentiate cover the mucosa.

In Colombia, South America, a well-characterized population has been studied. This population consisted of individuals who have chronic atrophic gastritis and who develop gastric cancer with very high incidence. Three findings were noted: an expansion of the proliferative compartment of epithelial cells; a grossly hyperproliferative state with excessive numbers of replicating cells in the gastric lining; and a failure of cells to undergo normal maturation [11]. Immature cells covered the surface of the stomach, with increased expression of an antigen in hyperproliferating cells that was normally decreased in maturing cells.

After surgical resection of part of the stomach to treat peptic ulcer disease, the susceptibility of individuals to develop gastric cancer in the remaining stomach may increase. Changes that develop in the gastric epithelium after partial gastrectomy included progressive expansions of the proliferative compartment of epithelial cells extending to the surface of the stomach with increasing dysplasia, and accumulations of increasing numbers of abnormally proliferating cells (e.g., 12).

Intestinal metaplasia also increased in the gastric mucosa of patients with cancer compared to individuals with gastric ulcer, suggesting that metaplasia may be a biomarker of precancerous disease. During development of the abnormal stage of cell differentiation known as metaplasia, which is associated with an increased frequency of stomach cancer, other changes developed in gastric epithelial cells. Differences in expression of intestinal enzymes in gastric mucosa have been used to classify metaplastic glands as "complete," i.e., containing all or most small intestinal enzymes, or "incomplete," i.e., with fewer enzymes expressed than in normal small intestinal mucosa [13]; the latter has been considered closer to dysplasia and carcinoma. In early and more mature metaplasia the neutral mucin of normal gastric cells was replaced by sialomucin (small intestinal type), while in advanced metaplasia sulphomucins of the colonic type were seen and considered a mark-

TABLE III. Biomarkers of Abnormal Differentiation of Gastrointestinal Epithelial Cells: Stomach

Organ	Biomarker	References
Stomach	Histopathology of superficial, chronic and atrophic gastritis	13
	Metaplasia of gastric epithelial cells	43 44 45 48 13
	Intestinal enzymes and mucin in metaplastic glands	45 46 47 48 13
	Aneuploidy of epithelial cell nuclei	49
	Differential protein localization	11
	Modified gene expression	50
	Modified response to growth factors	51

er of dysplasia. In metaplastic gastric epithelium normal gastric antigens also were lost; in well-differentiated lesions they were replaced by normal intestinal antigens; and in less well-differentiated lesions by embryonic antigens. In chronic atrophic gastritis, hyperproliferating epithelial cells increased the expression of an antigen that is normally decreased in maturing gastric cells.

More recently colonization of the gastric mucosa with Helicobacter pylori has been related to chronic antral and occasional body gastritis, possibly contributing to chronic atrophic gastritis. Recent epidemiological studies support a relationship to gastric carcinoma [14].

DISEASES OF THE LARGE INTESTINE

Benign colonic adenomas are believed to be an intermediate stage in the abnormal progression of normal colonic epithelial cells to carcinoma; the probability of a carcinoma developing in benign colonic adenomas increased directly as adenomas increased in size. In addition to an expansion of the proliferative compartment of epithelial cells in the colonic adenomas of familial polyposis, a complete shift of the entire proliferative region to the surface of the adenomas has been observed [15] (Figures 3, 4).

In diseases of the large intestine that lead to increased frequencies of human colorectal cancer, the flat colonic mucosa has also contained an expanded proliferative compartment (Table 4). The degree of expansion in the size of the proliferative compartment was smaller than in adenomas, identified by extensive counting of proliferating cells in the mucosa of human diseases; it has been observed in familial polyposis, in ulcerative colitis, and in individuals who have had sporadic adenomas, or

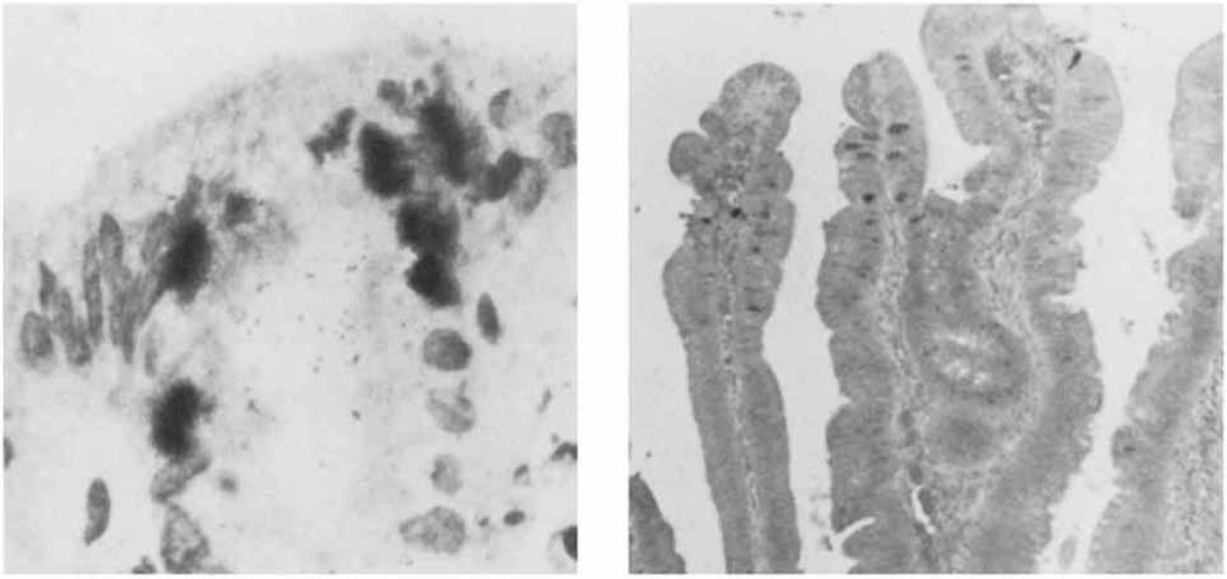

Fig. 3. Shift of proliferating epithelial cells to the surface of flat human colonic mucosa near an adenoma (left panel); and shift to the tips of human villous adenoma fronds (identified by Dr. G. Biasco), both with incorporation of [^{3}H]dThd and microautoradiography.

previous familial and non-familial colon cancers (Table 4). Similar findings occurred after administration of chemical carcinogens to rodents [1].

During the abnormal development of colonic epithelial cells in precancerous diseases (Table 5), blood-group-related antigens of the ABH and Lewis systems also become modified, with neosynthesis of ABH specificities appearing in tumor cells together with accumulation of precursor antigens. Increased expression of Lewis antigens, especially Y and extended Y determinants, has been found; the latter are not present in normal colonic mucosa and have a restricted pattern of distribution in normal tissues. LeY expression in polyps was further correlated with histological type and degree of dysplasia. Extended or trifucosyl LeY antigen expression also was limited to premalignant adenomatous polyps and was absent in nonpremalignant or hyperplastic polyps. Among adenomatous polyps extended LeY antigen expression tended to correlate with three known parameters of malignant potential: larger polyp size, villous histology and severe dysplasia. Therefore, in human colon the LeY hapten appeared to be an oncodevelopmental associated antigen, and extend-

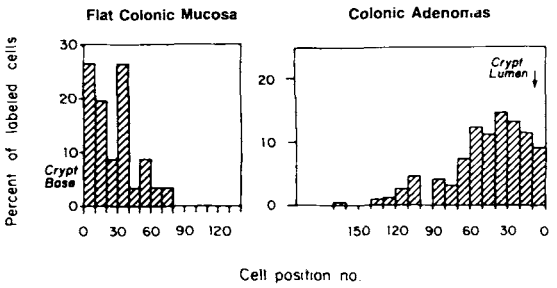

Fig. 4. Location of [^{3}H]dThd-labeled cells in lower part of colonic crypts in flat human colonic mucosa (left panel); and shift of entire proliferative compartment to surface of colonic crypts in human adenoma (right panel).

ed LeY antigens were believed to be highly specific markers for premalignancy and malignancy [16,17].

Further changes occur in colonic epithelial cells during abnormal differentiation and as neoplasms developed. Of major importance have been changes in gene structure and function including deletions of gene sequences as found in other instances of inherited predisposition to cancer. These genetic changes and modified gene expression have been found in the hereditary disease familial polyposis coli [18–22], and in sporadic and carcinogen-

induced colonic cancers [19,23–27]. In familial polyposis coli allelic deletion of the long arm of chromosome 5 was found [18,20,28] suggesting that the polyposis gene locus may encode a tumor suppressor gene similar to other inherited tumors. Such genes could function recessively with inactivation of both alleles (through deletion or mutation) required to have an effect.

Thus, in the progression of colonic cells to adenomas and carcinomas, multiple acquired genetic changes occur [19,20,22,24–27]. Other early changes have included mutation in a *ras* oncogene, and later changes probably include deletion of a segment of chromosomes 17 and 18 largely limited to advanced tumors [20,22]; not all tumors, however, show all of these, or the same sequence of, genetic alterations. Further, the dominant changes occurring in familial polyposis coli may not explain the effect of recessive changes also occurring in sporadic colonic cancer; and the loss of alleles at a single locus is not likely to explain all of the major genetic changes occurring in colon cancer [19,20,22,25]. The multiple alterations of gene structure and function noted above contribute to the abnormally increased proliferation and accumulation of abnormally differentiated colonic epithelial cells that occur; the early and late changes and some aspects of putative sequential development of these changes have been summarized by Vogelstein et al. (e.g., 20).

Thus, in diseases that lead to increased frequencies of cancer throughout the human gastrointestinal tract, expanding populations of abnormally proliferating epithelial cells have been found before the development of observable tumors. Throughout the entire gastrointestinal tract, therefore, the normal mucosa is comparatively quiescent in terms of cell proliferation, and fully mature cells are able to develop in order to normally function, and to cover and to protect the surface of the gastrointestinal tract. As cells progress through different stages of premalignancy, newer studies have begun to show lack of terminal differentiation of epithelial cells, modifications of gene structure and expression, and modified response of the cells to growth factors and tumor promoters that may further contribute to abnormal cell development.

TABLE IV. Expansion of the Proliferative Compartment of Epithelial Cells. Studies of Human Subjects: Large Intestine

Disease	References
Familial polyposis	52, 53, 54, 55, 56, 58
Sporadic adenomas	59, 68, 61, 62, 63, 64, 65, 66, 67
Colon cancer	68, 69, 57, 58, 63, 65, 67
Ulcerative colitis	70, 71, 72, 73, 74, 36, 75

APPLICATION OF BIOMARKERS TO STUDIES OF CANCER PREVENTION IN HUMAN SUBJECTS

It has recently been suggested that biomarkers of abnormal gastrointestinal cell proliferation and differentiation could assist studies in the field of cancer prevention [1]. Although genetic predisposition contributes to

TABLE V. Biomarkers of Abnormal Differentiation of Gastrointestinal Epithelial Cells

Organ	Biomarker	References
Large intestine	Development of colonic adenomas	86 76 77 78 79
	Histopathology of inflammatory diseases	76
	Cytokeratin expression in polyposis	80
	Blood group antigens	16 81 82 17
	Modified gene expression	23 91 19
	Modified response to growth factors	83 84 85

the evolution of gastrointestinal neoplasia, components of the ingested diet are believed to have a major influence on the incidence rates of both adenomas and colon cancer in human populations with widely differing frequencies of cancer in different parts of the world. Because of this, many studies have been carried out in animal models where the appearance of tumors can be measured over short time periods; these studies have indicated that specific dietary factors can inhibit the induction and development of a wide variety of tumors including those arising in the gastrointestinal tract.

Thus, new rationales for dietary intervention have emerged from epidemiological studies and from studies of animal models that might warrant evaluation in human populations. Since epithelial cell proliferation is increased in the colon, stomach, and esophagus of human subjects with increased susceptibility to gastrointestinal cancer before the appearance of tumors, analysis of patterns of gastrointestinal cell proliferation and differentiation have been considered for possible application to this problem.

The application of intermediate biomarkers to chemoprevention studies in human subjects can be considered in several stages, diagrammatically illustrated in Figure 5. It now appears possible to carry out initial pilot studies of putative chemopreventive regimens on small groups of human subjects, testing the effects of many nutritional and pharmacological interventions. A given study would evaluate whether a chemopreventive intervention regimen induced greater normalization of the cells, and whether the intervention modified the biomarkers studied in a direction characteristic of lower risk for neoplasia. In studies

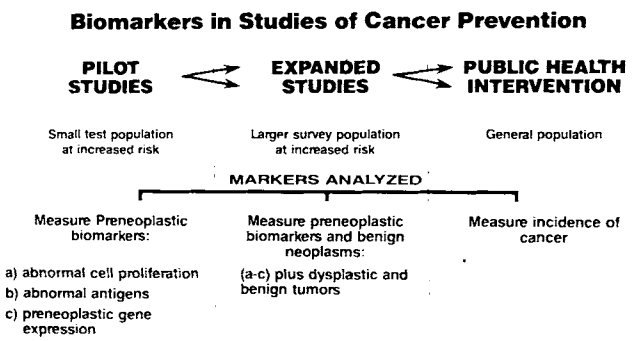

Fig. 5. Proposed application of intermediate biomarkers to study nutritional and pharmacologic chemopreventive interventions in human subjects in several stages, measuring possible modifications of cell proliferation and differentiation that occur and the eventual inhibition of tumor induction and development (from Reference 1).

of this type approximately twenty-five to fifty subjects have to be entered into the clinical trial for periods ranging from days to several months.

If a chemopreventive intervention successfully fulfilled biological, biochemical, and statistical criteria established for the pilot study, a second larger expanded study might be justified on more subjects for longer durations, measuring more advanced stages of neoplasia such as adenomas or dysplasias. In a second expanded study of this type larger numbers (usually hundreds) of subjects participating for longer durations (on the order of several years) are required to measure the effect of an intervention.

If the results of a second-stage expanded study fulfilled appropriate statistical criteria that had been established (e.g., decreased adenoma formation or decreased dysplasia), then consideration could be given to a third larger scale public health intervention trial over longer durations (many years) to measure the effect of the intervention on the development of cancer in a large human population of thousands to tens of thousands of individuals.

During this conference, the first meeting focusing on intermediate biomarkers or endpoints in cancer risk and their potential application to chemoprevention studies, participants will summarize the recent development of a large number of intermediate biomarkers measuring various aspects of cell proliferation, differentiation, and gene structure and expression. It is hoped that this effort will contribute to a comprehensive and critical review of the current state of this field, the continuing validation of these biomarkers in risk analysis, and the validation of their potential utility in the field of chemoprevention.

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