Concurrent Measurements of Carcinoembryonic Antigen, Glucosephosphate Isomerase, \(\gamma\)-Glutamyltransferase, and Lactate Dehydrogenase in Malignant, Normal Adult, and Fetal Colon Tissues

D. D. Munjal

Carcinoembryonic antigen and activities of glucosephosphate isomerase (EC 5.3.1.9), \(\gamma\)-glutamyltransferase (EC 2.3.2.2), and lactate dehydrogenase (EC 1.1.1.27) were measured in aqueous extracts of fetal, normal adult, and malignant human colon tissues. Fetal colon, as well as primary and metastatic colon tumor tissue, showed higher activities of these analytes than did normal adult human colon. Liver metastases of colon cancer gave the highest values, normal adult human colon the lowest. Statistically, these differences were more striking in the case of carcinoembryonic antigen and glucosephosphate isomerase than for \(\gamma\)-glutamyltransferase or lactate dehydrogenase. In contrast to the other markers, \(\gamma\)-glutamyltransferase activity was lower in fetal organs than in normal adult colon and colon tumors. These results are consistent with earlier observations that activities of these markers are significantly increased in the blood of patients with metastatic colon cancer.

Additional Keyphrases: tumor-related enzyme activity • cancer “markers” and their specificity

Carcinoembryonic antigen (CEA)\(^1\) content and the activity of several enzymes, especially glucosephosphate isomerase (GPI, also called phosphohexose isomerase), \(\gamma\)-glutamyltransferase (GGT), and lactate dehydrogenase (LD), have been determined separately in normal, benign, and malignant human tissues in several laboratories (1–5). Although none of these analytes is specific for cancer, their measurement in serum has been useful as an adjunct in the diagnosis and monitoring of cancer patients, when used with other information (6, 7). Plasma CEA and serum GPI, GGT, and LD are increased in patients with cancer or nonmalignant liver disorders (8, 9). Bodansky and others studied glycolytic enzymes in circulating blood and in a variety of neoplastic tissues and, already in 1954, considered GPI activity to be the best “index” of malignancy in cancer patients (10, 11).

The present study was undertaken to measure and compare concurrently the activities of CEA, GPI, GGT, and LD in extracts of fetal, normal adult, and malignant tissues, particularly of the colon.

Materials and Methods

Normal and both primary and metastatic colon tumor tissues were obtained at surgery or at autopsy (within 6–12 h of death), frozen, and stored at \(-70\, ^\circ\text{C}\) for one to six weeks before use. Organs from four fetuses at 6 through 16 weeks of gestation were obtained after abortion.

The tissues were extracted with ice-cold distilled water as described previously (12). Insoluble components and precipitates, if present, were removed by centrifugation.

CEA was measured by the \(Z\)-gel radioimmunoassay of Hansen et al. (13), with use of reagents supplied by Hoffmann-La Roche, Inc., Nutley, NJ 07110. Dilutions for use in preparing standard curves and sample dilutions for the CEA assay were made in ammonium acetate buffer (10 mmol/L, pH 6.5).

Enzymic activities in tissue extracts were measured by spectrophotometric procedures with reagent “kits” supplied by Worthington Biochemical Corp., Freehold, NJ 07728. We used the methods of Bueding and MacKinnon (14) for GPI, of Szasz (15) for GGT, and of Wacker et al. (16) for LD, with modifications made by Worthington Biochemical Corp.

To obtain values for CEA and the enzymes that fell within the range of sensitivities for these assays, we had to further dilute some samples. Results for CEA are expressed in micrograms per gram of protein, and for enzymes, as IUB units (U) per gram of protein. Protein determinations were made by the Folin phenol method (17), with bovine serum albumin as the reference standard. Statistical significance of the results was assessed by the method of Steel and Torrie (18).

Results

Table 1 shows our results for normal adult colon, primary colonic cancer, metastatic colon cancer, and four fetal tissues. The highest concentrations of CEA were in the liver metastases of colon cancer; primary colon cancer, as expected, had a higher CEA content than did normal adult colon. The same relationships were observed for GPI, GGT, and LD. Statistically, the increase in CEA and enzymic activities was significant (see Table 1). In the surgical and autopsy tissue specimens included in this study, no significant differences in enzymic activities were observed. Distribution of these four markers in fetal tissues was somewhat more variable. The concentration of CEA was highest in fetal intestine, followed in decreasing order by fetal lung, fetal brain, and fetal liver. Fetal intestine had the highest activity of GPI, followed by fetal brain, fetal liver, and fetal lung. No GGT activity was found in fetal lung or brain, but was observed in fetal liver and to a lesser extent in fetal intestine. LD activity was highest in fetal lung, followed by fetal intestine, fetal brain, and fetal liver.

The relationships among these substances in normal adult and fetal colon and primary and metastatic colon cancer are shown in Table 2. The ratios of CEA and GPI between the various malignant and normal tissues were quite similar; for GGT and LD, however, the ratios were highest between liver metastasis of colon cancer and normal adult colon, without a marked increase in the ratio of fetal intestine to normal adult colon, as occurred with CEA and GPI. It was apparent, nevertheless, that all four markers were consistently highest when colon cancer metastases to the liver were compared with normal adult colons.

\(^1\) Nonstandard abbreviations used: CEA, carcinoembryonic antigen; GPI, glucosephosphate isomerase (EC 5.3.1.9); GGT, \(\gamma\)-glutamyltransferase; LD, lactate dehydrogenase (EC 1.1.1.27); NAD*, oxido-reductase.

Received June 16, 1980; accepted Aug. 8, 1980.
I then compared values for the four markers in aqueous extracts of normal and neoplastic colon tissues removed from the same patients. In one such case, where normal and both primary and metastatic colon carcinoma tissues were available (also three other cases where normal colon and primary colon carcinoma tissues were available), the activities of all four markers were highest in the liver metastases of colon cancer, intermediate in the primary colon tumor, and lowest in the patients with apparently normal colon (Table 3). These findings are thus consistent with the relationships found in such tissues obtained from different patients (Table 1).

**Discussion**

The simultaneous monitoring of several markers believed to reflect tumor activity is gaining in clinical attention and use, and the view that a combination of monitors increases clinical predictability is receiving support from various lines of investigation (6, 7, 19). In a previous study, I and others found that blood content of CEA, GPI, GGT, and LD was increased in the serum of patients with cancers of the gastrointestinal tract, breast, and lung (6, 7, 20); similar increases were also reported in tumor tissue extracts (21, 22). Other workers have reported 2.5-fold higher activities of glycolytic enzymes in primary colon carcinomas than in normal colonic mucosa (22, 23). Values for several glycosyltransferases also have been found to be above normal in the sera of patients bearing mammary or colonic carcinomas (1, 24). Moreover, all these enzymes, like CEA, are increased in the serum of patients suffering from various liver diseases; this may be due in part to hepato-cellular damage accompanied by compensative hyperplasia. Shonk et al. (22) compared the patterns of glycolytic enzymes in colon carcinomas and hepatomas; they found similar patterns in different colon carcinomas, whereas hepatomas were quite variable. Hilf et al. (3) reported enzyme activities in normal breast tissue, fibrocystic disease, and in infiltrating ductal carcinoma. GPI activity in fibrocystic disease was threefold that in normal breast tissue, and eight- to 10-fold higher than normal in breast-cancer tissue. The cellular source of this increased enzymic activity was not evident.

Goldman et al. (2) reported LD activities in various human neoplastic tissues (colon, stomach, lung, breast, pancreas, ovary, thyroid, prostate, kidney, brain, and uterus), benign tumors, and normal control tissues. Their results showed consistently higher LD activity in the malignant tumors,
whereas benign tumors gave results similar to those of the normal tissues. Similarly, Hoch-Ligeti et al. (4) found higher LD activity in primary and metastatic tumors than in normal tissues from the same individuals. They also observed that LD activity was increased in tumor-free organs of patients with widespread metastases, compared with the corresponding organs of tumor-free patients. These findings suggested to them that the increase of LD activity in the organs of patients with disseminated malignant tumors reflected metabolic changes in the host, although the presence of micrometastases cannot be ruled out entirely.

Currently available information on tumor markers and enzymes indicates that no single enzyme or combination of enzymes, or, for that matter, antigens, is a reliable index to any particular cancer type, except for substances known to be a product indigenous to the cell type in question—for example, choriogonadotropin in choriocarcinoma. Because combined data on CEA, GPI, GGT, and LD are becoming of increased clinical interest in monitoring cancer patients, I undertook the present study. The observation that values for these substances are highest in colon cancer metastatic to the liver supports clinical evidence that these markers are of greatest use in reflecting disease activity in patients with liver metastases (6, 7). These markers have also been found to be above-normal in the sera of jaundiced patients, alcoholics, and patients with other benign liver diseases (20, 25). However, aqueous tissue extracts of primary colon cancers have increased values for these markers, and so such increases in cancer patients may better reflect activity in malignant tissues than liver malfunction. Nevertheless, in some patients with liver metastases, liver changes such as compensatory hyperplasia could also contribute to increased circulating activities of these enzymes. In the case of CEA, liver malfunction owing to secondary tumor deposits could also result in increased circulating amounts of this antigen if hepatic metabolism of CEA is compromised.

These findings are also consistent with recent observations made on circulating CEA and GPI in hamsters bearing the GW-39 human colon carcinoma (12, 26). Amounts of both markers in blood correlated with the growth of the tumor in the hamster, suggesting that these substances could be used as indices of tumor growth. Also, a decrease in circulating CEA and GPI after complete resection of GW-39 tumors further confirmed that both these markers in the circulating blood reflect the proliferative behavior and (or) mass of tumor in the animal (26). When tissue antigen and enzyme content were measured, CEA and GPI values in colonic tumors much exceeded values in normal human colon or normal hamster organs (12).

The results reported here indicate that the increased amounts of CEA, GPI, GGT, and LD found in patients with colon cancer, particularly those with liver metastases, are derived from increased concentrations in the tumors. However, the current study has not resolved whether any one or a combination of these markers are in any way specifically increased in colon cancer. This would require measurement of these substances in several tumor types. Interestingly, of the various enzymes examined, GPI was the most increased in colon carcinoma, either primary or metastatic. A decision as to whether qualitative differences also exist for GPI in various tumors, and between colon cancer and normal adult or fetal human colon, must await isolation, purification, and characterization studies.

This work was done at Mallory Institute of Pathology Foundation, Boston City Hospital, Boston, MA, and the Department of Pathology, University of Kentucky, Lexington, KY. It was supported in part by NIH General Research Support Grant RR 05374 to the University of Kentucky, Lexington, KY. I thank Stephen Lewandowski and Patricia Moison-Thomas for technical assistance, Fred S. Ruland, of the Statistics Laboratory, Ohio State University, for analysis of statistical significance, and Worthington Biochemical Corporation for providing reagent “kits” for enzymes. I am particularly grateful to Dr. N. Zamcheck of Mallory Institute of Pathology Foundation for this initial support of this project, and for providing some of the earlier tumor specimens. I also thank Dr. D. M. Goldenberg of the Department of Pathology, University of Kentucky, for his help in getting fetal tissues.

References
21. Munjal, D., and Goldenberg, D. M., Quantitation of carcinoembryonic antigen (CEA), glucosephosphate isomerase (GPI),...


