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Purine Enzymology of Human Colon Carcinomas

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ABSTRACT

The purpose of this study was to elucidate the purine enzymic programs of human primary colorectal carcinomas. Marked alteration in the enzymology of the human colon neoplasm clearly distinguished it from that of the normal colon mucosa. In the human colon mucosa, the activities of ribonucleotide reductase, inosine phosphate dehydrogenase, formylglycinamidine ribonucleotide synthetase, guanosine phosphate synthetase, and amidophosphoribosyltransferase were 0.042, 5.2, 5.6, 8.2 and 36.0 nmol/h/mg protein, respectively, and in the colon carcinomas the activities increased to 755, 575, 295, 280, and 294% of the normal values. The activities of the salvage enzymes, adenine and hypoxanthine-guanine phosphoribosyltransferases, were 310, 249, and 602 nmol/h/mg protein, respectively, whereas in the tumors, only the activity of adenine phosphoribosyltransferase was increased (2-fold). The markedly higher absolute enzymic capacity for salvage in the tumors accounts, in part at least, for the lack of chemotherapeutic success of inhibitors of enzymes of *de novo* synthesis that have been used in the clinical treatment of colorectal carcinomas. Combinations of inhibitors of *de novo* biosynthesis and blockers of the salvage enzymes or of salvage transport (*e.g.*, dipyridamole) should improve the chemotherapy of colon neoplasms. Since in the colon carcinoma the activities of glutamine-utilizing enzymes (guanosine phosphate and formylglycinamidine ribonucleotide synthetase and amidophosphoribosyltransferase) were markedly increased, and the glutamine concentration was decreased (50%), treatment with an antiglutamine agent (*e.g.*, acivicin) should be of relevance. Since the activity of ribonucleotide reductase, the rate-limiting enzyme of nucleic acid biosynthesis, was markedly increased in the colon neoplasms, combination chemotherapy might include drugs against this enzyme.

INTRODUCTION

Colorectal carcinoma is one of the most common solid tumors in humans, yet current clinical chemotherapy has failed to provide curative results. For the rational design of selective chemotherapy, it should be helpful to elucidate the biochemical program of human colon neoplasms (23). Previous work in this laboratory determined the pattern of enzymic imbalance in chemically induced transplantable mouse colon tumors (28) in a spectrum of transplantable colon carcinoma xenografts carried in athymic (nude) mice (27) and the enzymology of carbohydrate and pyrimidine metabolism of primary human colon carcinomas (4). The present work seeks to answer the following questions: (*a*) is there a biochemical pattern in purine metabolism in human colorectal neoplasia that distinguishes it from normal human colon mucosa? (*b*) does an understanding of the integrated program of purine enzymology of human colon tumors account for the current problems in the chemotherapy of these tumors and (*c*) does it suggest new drug treatment approaches for the clinical therapy of colorectal neoplasia?

MATERIALS AND METHODS

Clinical Materials. In our series of 6 cases (all males), the colon adenocarcinomas were obtained at operations from patients undergoing surgical resection at the hospitals of Indiana University School of Medicine. After tissue samples were taken for pathology, the specimens were placed in beakers embedded in ice and within 30 min were transferred to this laboratory. The tissues were processed immediately, as reported earlier (4). For each case, different parts of the tumor were sampled to provide multiple assays from potentially different populations of tumor cells. The mucosa of the uninvolved and histologically normal colon of the same patient was the control for the carcinoma. Concurrently with the human samples, livers of normal male Wistar rats weighing 190 to 210 g were processed. Comparison of rat liver enzymatic activities with our previous results provided internal controls for the enzyme assays. The various considerations in the evaluation of biochemistry of human tumors were outlined elsewhere (26).

Tissue Preparation and Biochemical Assays. The tissues were excised, cleaned, and prepared for biochemical analyses as cited earlier (23, 27). Protein concentration was determined by a standard method (10). The concentrations of glutamine and glutamate were measured as reported (19). All enzyme assays were done in the 100,000 × *g* clear supernatant fluid. Previously reported procedures were used for the preparation of extracts and measurement of activities of ribonucleotide reductase (EC 1.17.4.1) (20), IMP dehydrogenase (EC 1.2.1.14) (8, 17), FGAM[2] synthetase (EC 6.3.5.3) (6), GMP synthetase (EC 6.3.5.2) (3), amidophosphoribosyltransferase (EC 2.4.2.14) (16), adenine phosphoribosyltransferase (EC 2.4.2.7), and hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8) (13). All enzyme assays were adapted for the conditions required for optimum activity in the human colon mucosa and tumor systems, and the activities were determined under linear kinetic conditions.

Expression and Statistical Evaluation of Results. Specific activities were expressed as nmol substrate utilized per h per mg protein. The means and SEs of the colon tumor activity were compared with those of the normal colon mucosa using the *t* test for small samples. Differences between means yielding a *P* of less than 5% were considered statistically significant.

RESULTS AND DISCUSSION

Patient Material. Histological grading of the 6 specimens determined that one was well differentiated, 3 were moderately differentiated, and 2 were poorly differentiated. Because of the

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[2] The abbreviations used are: FGAM, formylglycinamidine ribonucleotide; TAD, thiazole-4-carboxamide adenine dinucleotide; the trivial name used is: tiazofurin, 2-β-D-ribofuranosylthiazole-4-carboxamide.

small number of cases, a statistical evaluation did not detect significant differences between the biochemistry of the 3 grades of neoplasms; therefore, the data of the enzyme assays from all neoplasms were combined in this work. The surgical samples were from the following areas of the colorectum: 2 from cecum; one from the descending colon; 2 from the sigmoid; and one from the rectum.

Enzymic Programs of Human Colon Mucosa and Colon Tumor. The enzymes were selected for these studies because of their strategic roles in the various metabolic pathways (23) and for comparison with changes in enzymic patterns observed in rat hepatomas and sarcomas (23, 25) and human colon carcinoma xenografts carried in nude mice (27). Another reason for the selection was that all 5 of the enzymes of *de novo* purine synthesis are targets of antimetabolites. Therefore, determining their activities should have relevance for the design of chemotherapy in colon carcinomas.

In Table 1, the key enzymes of purine biosynthesis in normal human colon mucosa and in colon carcinomas are listed in order of increasing specific activities in the *de novo* pathway. The activity of the ribonucleotide reductase which in normal liver was at least 2 orders of magnitude lower than that of IMP dehydrogenase (23) was also very low in the normal human colon mucosa, being 0.042 nmol/h/mg protein. IMP dehydrogenase in rat liver was the rate-limiting enzyme of guanylate biosynthesis (23, 24) and the enzyme with the lowest activity in purine metabolism (24, 25); this was also the case in human colon mucosa. The activity of amidophosphoribosyltransferase (36 nmol/h/mg protein) was 6.9-fold higher than that of IMP dehydrogenase. The activities of adenine phosphoribosyltransferase, hypoxanthine phosphoribosyltransferase, and guanine phosphoribosyltransferase were 310, 249, and 602 nmol/h/mg protein, respectively. Thus, the activities of these salvage enzymes were 47.9- to 115.7-fold higher than that of IMP dehydrogenase.

In the colon carcinomas, the activities of enzymes of the *de novo* pathway, ribonucleotide reductase, IMP dehydrogenase, FGAM synthetase, GMP synthetase, and amidophosphoribosyltransferase were increased to 755, 575, 295, 280, and 294% of that of the normal colon mucosa. The activities of the salvage enzymes behaved differently in the carcinoma. The activity of adenine phosphoribosyltransferase in the tumors was increased 2-fold; however, hypoxanthine phosphoribosyltransferase and guanine phosphoribosyltransferase activities were unchanged. Since in the normal colon mucosa these salvage enzymes were present in activities one to 2 orders of magnitude higher than those of the enzymes of the *de novo* pathways, the small changes observed in their activities in colon carcinomas were not unexpected on the basis of similar experience in hepatomas of different growth rates in the rat (13).

The concentration of glutamine was also determined because it has a bearing on the activities of the glutamine-utilizing enzymes and because of its possible role in the use of antiglutamine agents in chemotherapy. The results showed that the glutamine concentration of the normal mucosa (1.6 μmol/g) decreased to 50% in the colon carcinoma. By contrast, the concentration of L-glutamate (1.0 μmol/g) was unchanged in the tumor.

Pattern of Enzymic Activity Changes in Individual Samples of Human Colon Carcinomas. Table 2 shows that in all colon tumor samples tested the activities of ribonucleotide reductase, IMP dehydrogenase, FGAM synthetase, and amidophosphoribosyltransferase were elevated as compared to values of the normal mucosa. By contrast, the GMP synthetase activity was increased in 16 of 22 samples and decreased in 6 samples. Among the salvage enzymes, adenine phosphoribosyltransferase was elevated in 8 of 8 samples, whereas hypoxanthine-guanine phosphoribosyltransferase activities were higher than normal in 5 and decreased in 3 of the 8 samples.

Stringency of Linkage of Enzyme Activities and L-Glutamine Content with Neoplasia in Human Colon. The increased activities observed for ribonucleotide reductase, IMP dehydrogenase, FGAM synthetase, amidophosphoribosyltransferase, and adenine phosphoribosyltransferase occurred in 100% of the samples. Therefore, these alterations in gene expression appear to be stringently linked with the neoplastic transformation of the human colon, and they might be used as markers for colon neoplasia. By contrast, the activities of GMP synthetase and hypoxanthine-guanine phosphoribosyltransferases were increased in only 73 or 63% of the cases examined; these would not be useful markers of neoplasia in this tumor.

The decrease of L-glutamine also occurred in 100% of the samples. The depletion of L-glutamine content was to 50% of that of control normal human colon mucosa. In comparing the

Table 1

Purine enzymic programs of normal human colon and colon adenocarcinoma

Enzymes and amino acids	EC No.	No. of patients	No. of samples	Enzymic activities (nmol/h/mg protein)		
				Normal colon mucosa	Carcinoma	% of normal colon
De novo synthesis						
Ribonucleotide reductase	1.17.4.1	4	12	0.042 ± 0.007[a]	0.317 ± 0.052	755[b]
IMP dehydrogenase	1.2.1.14	5	18	5.2 ± 0.7	29.9 ± 10.7	575[b]
FGAM synthetase	6.3.5.3	4	13	5.6 ± 0.4	16.5 ± 1.5	295[b]
GMP synthetase	6.3.5.2	5	22	8.2 ± 0.6	23.0 ± 3.8	280[b]
Amidophosphoribosyltransferase	2.4.2.14	4	10	36.0 ± 6.4	106 ± 7.7	294[b]
Salvage synthesis						
Adenine phosphoribosyltransferase	2.4.2.7	3	8	310 ± 31	647 ± 106	209[b]
Hypoxanthine phosphoribosyltransferase	2.4.2.8	3	8	249 ± 39	274 ± 54	110
Guanine phosphoribosyltransferase	2.4.2.8	3	8	602 ± 128	604 ± 117	100
Amino acids, μmol/g						
L-Glutamate		4	15	1.0 ± 0.05	0.9 ± 0.1	90
L-Glutamine		4	15	1.6 ± 0.1	0.8 ± 0.1	50[b]

[a] Mean ± SE of 4 or more samples.
[b] Significantly different from normal colon mucosa, at $P < 0.05$.

Table 2
Pattern of enzyme activity changes in individual samples of human colon neoplasia

Enzymes and amino acids	No. of samples tested	Specific activity in tumor, no. of cases in which tumor activity was: Increased (>120% of control)	Unchanged (80–120% of control)	Decreased (<80% of control)
De novo synthesis				
Ribonucleotide reductase	12	12	0	0
IMP dehydrogenase	18	18	0	0
GMP synthetase	22	16	0	6
FGAM synthetase	13	13	0	0
Amidophosphoribosyltransferase	10	10	0	0
Salvage synthesis				
Adenine phosphoribosyltransferase	8	8	0	0
Hypoxanthine phosphoribosyltransferase	8	5	0	3
Guanine phosphoribosyltransferase	8	5	0	3
Amino acids				
L-Glutamate	15	6	0	9
L-Glutamine	15	0	0	15

50% decrease in L-glutamine concentration in colon carcinoma with that of rat hepatomas of different growth rates, the decrease would be in the range of that observed for medium growth rate hepatomas (19). This is in line with the relatively slow growth rate generally assumed for human colon carcinoma. The extent of increases in specific activities of the ribonucleotide reductase (20), IMP dehydrogenase (8), and GMP and FGAM synthetases (3, 6) is also in line with those found in rat hepatomas of slow or intermediate growth rates (23).

Selective Advantages Conferred to Colon Carcinomas by the Enzymic and Metabolic Imbalance. As the results showed (Table 1), in the colon carcinomas there was a markedly elevated capacity of the first committed enzyme of *de novo* IMP biosynthesis, amidophosphoribosyltransferase. The increased capacity for *de novo* IMP synthesis was also detected in the increase of one of the subsequent enzymes of *de novo* biosynthesis, FGAM synthetase. The rise in the *de novo* synthetic capacity for guanylate production from IMP was indicated by the increased activities of IMP dehydrogenase and GMP synthetase. The elevated ribonucleotide reductase activity signals a heightened potential for the biosynthesis of deoxynucleotides.

It is impressive that in the normal colon mucosa the salvage enzymes of purine synthesis are present in orders of magnitude higher activities (249 to 602 nmol/h/mg protein) than those of the enzymes of the *de novo* pathway (0.042 to 36 nmol/h/mg protein) and that adenine phosphoribosyltransferase activity was further increased in the colon tumors. In the colon neoplasms, the elevated activities of the *de novo* enzymes of purine biosynthesis and the high activities of the salvage enzymes should provide an increased capacity for purine biosynthesis. This should confer selective advantages to the colon carcinoma cells. Similar observations indicating increased activities of *de novo* and salvage enzymes of pyrimidine biosynthesis were reported in human colon carcinoma (4). The integrated stepped-up activity for purine and pyrimidine biosynthesis and the high activities of the salvage enzymes should provide a formidable capacity for nucleic acid biosynthesis in the colon carcinoma.

This integrated pattern of biochemical imbalance might account for the failure of current antimetabolite treatment of colon tumors, since even complete blocking of the *de novo* pathway cannot be successful for chemotherapy because of the high salvage enzymic capacity of human colon carcinomas. However, the identification of the selective advantages conferred on cancer cells by the biochemical imbalance should mark out potentially sensitive targets for enzyme pattern-targeted chemotherapy (4, 21–23, 27, 28).

Indications for the Design of Anticancer Drug Treatment from the Enzymic Imbalance of Human Colorectal Carcinoma. Our results (Table 1) showed that activities of enzymes of glutamine utilization (amidophosphoribosyltransferase, FGAM synthetase, and GMP synthetase) were increased in most or all the samples examined (Table 2). These observations indicate that utilization of antiglutamine agents such as acivicin might be helpful in the treatment of human colon tumors, since the L-glutamine content was decreased in the colon carcinomas. However, as we have pointed out, whereas acivicin is a potent competitive inhibitor (2, 5, 9, 11, 14, 15, 18, 30, 32) and inactivator of key enzymes of glutamine utilization *in vitro* and *in vivo* (2, 5, 11, 23, 30–32) including carbamoyl-phosphate synthetase II in human colon carcinoma (18), lasting successful chemotherapy is not to be expected because of the presence of the powerful pyrimidine and purine salvage pathways (23, 30). Therefore, if acivicin is used, it should be in combination chemotherapy with an inhibitor of salvage transport, *e.g.*, dipyridamole, as we have suggested (2, 5, 12, 23, 29, 30, 35, 36). Recently, evidence was obtained that acivicin and dipyridamole in human colon carcinoma cell lines is indeed a promising combination (7, 33), and such combination chemotherapy is now in Phase I trials (34).

Because of the increased activity of IMP dehydrogenase in this tumor, an inhibitor of this enzyme, *e.g.*, tiazofurin, may be considered. However, because of the powerful activity of guanine phosphoribosyltransferase reported in this paper (Table 1), the use of tiazofurin probably should also be coupled with a blocker of guanine transport, *e.g.*, dipyridamole, or if available, an inhibitor of this salvage enzyme activity. It is important that tiazofurin action depends on the synthesis of its active metabolite, TAD. Therefore, studies of the activities of the synthetic and catabolic enzymes of TAD and their ratios along with determination of TAD content after tiazofurin administration should also provide an indication for the possible use of this antiguanylate drug (1).

In conclusion, the following answers are provided to the questions raised in the "Introduction": (*a*) there are marked purine enzymic differences between normal and neoplastic human colon cells; (*b*) this biochemical imbalance might account, in part at least, for the failure of chemotherapy of colon cancer because of the powerful activities of purine and pyrimidine (4, 23, 35, 36), salvage enzymes which might circumvent the inhibitions provided by drugs of the *de novo* nucleic acid synthetic pathways. The presence of a large noncycling growth fraction in primary human colon carcinomas contributes to the low sensitivity of these tumors to anticancer drug action; and (*c*) concrete suggestions as to how one might proceed from these enzymic indications and chemotherapeutic approaches involving inhibitors of the *de novo* pathways (*e.g.*, acivicin) and of salvage transport (*e.g.*, dipyridamole) were brought up for consideration.

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