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ACTIVITIES OF PURINE-METABOLIZING ENZYMES IN HUMAN COLON CARCINOMA CELL LINES AND XENOGRAFT TUMORS*

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Abstract—The activities of eleven enzymes of purine metabolism have been determined in extracts of four human colon carcinoma cell lines (clones A and D of the heterogeneous DLD-1 line, HCT-15 and DLD-2) in culture and as xenografts in nude mice. Activities of the enzyme adenine phosphoribosyltransferase (APRT), hypoxanthine phosphoribosyltransferase (HPRT), adenosine kinase, 5′-deoxy-5′-methylthioadenosine (MTA) phosphorylase, adenylate (AMP) kinase, guanylate (GMP) kinase, and nucleoside diphosphokinase (NDP kinase) changed little from line to line *in vitro*. Adenosine deaminase (ADA) activity varied more than 5-fold between HCT-15 and clone A cells. Purine nucleoside phosphorylase (PNP) activity was about 3-fold higher in DLD-2 cells than in the other three lines. Guanine deaminase (guanase) activity was 4- to 6-fold higher in clone A and D cells than in HCT-15 and DLD-2 cells. Xanthine oxidase was not detected in any of the lines grown *in vitro* or *in vivo*. The activity of APRT was highest in clone D xenografts. HPRT was lowest in DLD-2 tumors. ADA activity in DLD-2 tumors was more than 2-fold elevated over that for clone A and HCT-15 xenografts. PNP activity in clone A and HCT-15 xenografts was lower than that for clone D or DLD-2 tumors. Guanase was lower in HCT-15 and DLD-2 tumors than in those of clones A and D. Clone D tumor GMP kinase activity was elevated more than 4-fold above that for the other three tumors. The activity of NDP kinase was highest in clone D tumors. MTA phosphorylase activity was similar in all four xenografts. In general, the activity of a given enzyme was similar in cells growing in monolayer culture and as xenografts in nude mice (37/44 comparisons). Enzyme activities were also measured in normal human colon and compared to those in the xenografts. Where significant differences were seen, the values for normal tissue were almost always lower than those of the tumors. The striking similarities between the *in vitro* and *in vivo* enzymic profiles for each of these four human colon carcinoma lines indicate that, at least on this basis, responses of these cells to purines and purine analogs in the two-dimensional monolayer culture system should be predictive of *in vivo* responses.

Although purine analogs have been used clinically to treat various forms of leukemia [1], they have been shown to be largely ineffective against human solid tumors. In the few trials of this class of agents reported to date, the purine analog, 6-thioguanine, in combination with the glutamine antagonist, azaserine, was of limited value in the treatment of multiple myeloma [2, 3] and several solid neoplasms [4]. Similarly, a few positive responses have been reported for head and neck tumors treated with 8-azaguanine [5]. 6-Mercaptopurine has shown marginal activity as a single agent against renal cell carcinoma [6], ovarian carcinoma [7], and malignant melanoma [8].

The reasons for the ineffectiveness of purine analogs as chemotherapeutic agents against solid tumors have not yet been elucidated. In fact, basic information on normal purine metabolism in such tumors is lacking, a major deterrent in the past being the unavailability of suitable experimental systems. Recently, three human colon carcinoma cell lines, designated DLD-1, DLD-2 and HCT-15, have been established in our laboratory from primary tumors [9]. One of these lines, DLD-1, is heterogeneous and has been cloned to provide two distinct subpopulations, clone A and clone D [9]. DLD-1 clones A and D and HCT-15 cells have been used in the investigation of differentiation and heterogeneity of human colon carcinoma, and in pharmacological studies [10–13]. DLD-1 and its derived clones, and the other two parent lines, DLD-2 and HCT-15, constitute a spectrum of colon cancer cell lines that offers a promising system for drug evaluation. All of these lines are tumorigenic [9], and inoculation of nude mice with the different cell types results in tumors ranging histologically from poorly-differentiated carcinomas (clone A) to well-differentiated colonic adenocarcinomas with significant mucus production (DLD-2). Thus, the model provides a series of distinct human colon cancer cell lines and their corresponding xenograft tumors for testing analogs in culture and *in vivo*. The fact that chemotherapy

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has been relatively ineffective against human colorectal cancer [14] makes this system even more relevant for the testing of a class of drugs that to date has not received a systematic evaluation against this group of tumors.

To establish this system as a model for testing the antitumor activities of purine analogs, it is necessary to measure the activities of the key anabolic and catabolic enzymes of purine metabolism. Comparison of levels of activities of enzymes in cultured cells and in the corresponding xenografts is also necessary to indicate whether extending the results of culture screening to animal testing is biochemically sound, with reference to cancer cell target or drug-metabolizing enzymes. This report describes the measurement of enzymes of purine metabolism in four human colon cancer cell lines. These lines represent the spectrum of histological variation found in human colon cancer. Portions of this work have been presented in preliminary form [15]. The reactions catalyzed by the enzymes examined in this study are illustrated in Fig. 1.

METHODS

Materials. The following chemicals were obtained from commercial sources: 5-phosphoribosyl-1-pyrophosphate, adenine, hypoxanthine, 5'-deoxy-5'-methylthioadenosine, and milk xanthine oxidase, Grade III (Sigma Chemical Co., St. Louis, MO); adenosine, AMP, ADP, ATP, and IMP (PL Biochemicals, Milwaukee, WI); and dithiothreitol (Calbiochem-Behring, La Jolla, CA). Samples of 2'-deoxycoformycin [(R)-3-(2-deoxy-D-erythro-pentofuranosyl)-3,6,7,8-tetrahydroimidazo [5,4-d] [1,3] diazepin-8-ol] were provided by Dr. H. W. Dion of Parke-Davis, Detroit, MI, and by Dr. John Douros of the National Cancer Institute, Bethesda, MD. The following radiochemicals were purchased from commercial sources: [8-^{14}C]adenine and [8-^{14}C]hypoxanthine (Schwarz/Mann, Orangeburg, NY); and [8-^{14}C]adenosine (New England Nuclear Corp., Boston, MA).

Cell lines. The establishment at the Roger Williams Cancer Center of the human colon carcinoma cell lines HCT-15 and DLD-1 and the isolation of clones A and D from the heterogeneous DLD-1 line have been reported previously [9]. Clone A and clone D cells produce, in nude mice, poorly-differentiated and moderately-differentiated adenocarcinomas respectively. Inoculation of nude mice with HCT-15 cells produces well- to moderately-differentiated adenocarcinomas. A third human colon cancer cell line has been established recently and has been designated DLD-2; this line appears homogeneous in tissue culture. Cells from this line grow more slowly than do the other colon cancer cells (doubling time of 48 hr compared to about 20 hr for the other lines). Also DLD-2 cells do not grow in soft agar, whereas the other cell lines are clonogenic in semi-solid medium. Tumors produced by inoculation of nude mice with DLD-2 cells are characterized histologically as well-differentiated, mucus-secreting, adenocarcinomas; this histology closely resembles that of the original human carcinoma of the colon.

Preparation of homogenates. Cells were grown in 100 mm tissue culture dishes (Falcon Plastics, Oxnard, CA) until cultures were almost confluent; fifteen plates were prepared for each cell type. The cell culture medium (RPMI-1640 supplemented with fetal calf serum and antibiotics) and culture techniques have been described previously [9]. The medium was decanted and each plate was rinsed twice with 10 ml of 0.9% NaCl solution; then 1 ml of potassium phosphate buffer (0.1 M; pH 7.4) was added to the first dish and the cells were removed by scraping with a rubber policeman. The slurry was transferred with a Pasteur pipette to the next dish and the process was repeated until the cells from the entire set of plates had been harvested. The cell suspension was kept at 4°. Then 0.5 ml of buffer was added to the first dish and the sequential rinsing and scraping were repeated for all dishes. This wash volume was then added to the initial slurry, and the entire volume was transferred to a 15 ml homogenizer and the cells were broken with 15 strokes.

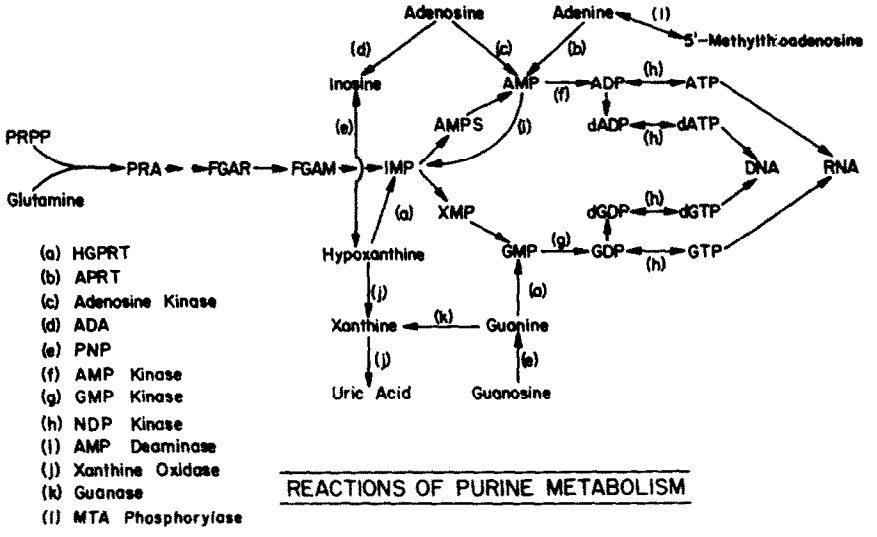

Fig. 1. Reactions of purine base, nucleoside, and nucleotide metabolism.

The homogenate was centrifuged for 1 hr at 100,000 *g* in a Beckman L2-65 ultracentrifuge at 5°. The supernatant fluid was removed with a Pasteur pipette and was used immediately in the enzyme assays. The pellet was also used for determinations of adenosine kinase activity.

Tumors (see below) were removed from nude mice, and each was homogenized in 5–6 ml of potassium phosphate buffer (0.1 M; pH 7.4) at 5°. The homogenates were processed in a manner similar to those prepared from cultured cells.

Human colon carcinoma xenografts. Athymic, nude mice bearing the nu/nu genotype on an outbred Swiss background are bred and maintained in the Roger Williams General Hospital Animal Care Facility. Nude mice were inoculated s.c. with 5×10^6 cells harvested from human colon carcinoma cell cultures, as reported previously [9]. When tumors had grown to a size of about 300–600 mg, they were removed and processed (see above) for enzyme analyses.

Normal colon tissue. Normal colonic mucosa was obtained from two patients in the normal course of operation for diverticulitis at the Roger Williams General Hospital. Neither patient had evidence of cancer and appropriate informed consent was obtained. These tissues were processed for enzyme analyses as described above.

Enzyme assays. All enzyme assays were performed at room temperature unless indicated otherwise. APRT* and HPRT activities were determined by a modification of the radiochemical method of Kelley *et al.* [16]. Reaction mixtures (500 μl) contained 0.1 M Tris–acetate buffer, pH 7.4; 5 mM $MgCl_2$; 1 mM 5-phosphoribosyl-1-pyrophosphate; extract (25 or 50 μl); and either 0.1 mM [8-^{14}C]adenine (sp. act. 11.0 mCi/mmole) or 0.1 mM [8-^{14}C]hypoxanthine (sp. act. 9.9 mCi/mmole). At appropriate times after initiation of the reaction by addition of cell extract, aliquots (100 μl) of the reaction mixtures were removed and added to 20 μl of 4 M formic acid to terminate the reactions. Aliquots (10 μl) of the formic acid extracts were spotted on microcrystalline cellulose thin-layer chromatography sheets (J. T. Baker Chemical Co., Phillipsburg, NJ) that had been spotted previously with the appropriate nonradioactive base and nucleotides as carriers. Nucleotide products were separated from the precursor base by development of the sheets in aqueous 5% Na_2HPO_4. Following development, areas corresponding to the base and nucleotides were visualized by u.v. (254 nm), cut out, and counted in a toluene-based liquid scintillation system [15 g Omnifluor (New England Nuclear Corp.) in 1 gal scintillation grade toluene]. Nucleotide formation was linear with respect to both time and protein concentration under these conditions.

The activity of adenosine kinase was determined by a radiochemical assay. The reaction mixture (500 μl) contained: 100 mM Tris–HCl buffer, pH 7.0; 50 μM [8-^{14}C]adenosine (sp. act. 10 mCi/mmole); 0.5 mM $MgCl_2$; 1 mM ATP; 10 mM NaF; 5 mM dithiothreitol; 2 μM 2′-deoxycoformycin and up to 250 μl of extract. The extract was preincubated for 10 min in the presence of the adenosine deaminase inhibitor 2′-deoxycoformycin before the reaction was started by the addition of labeled adenosine. Aliquots (50 μl) were withdrawn at appropriate time intervals and added to 10 μl of formic acid at 4° to terminate the reaction. After centrifugation for 10 min at 15,000 *g*, 10 μl samples of the supernatant fractions were spotted on thin-layer chromatography sheets (see above) spotted previously with unlabeled adenosine and AMP as carriers. Sheets were developed in 95% ethanol–1 M ammonium acetate, pH 7.5 (7:3). Areas corresponding to adenosine and adenine nucleotides were visualized by u.v. light (254 nm), and their constituent radioactivity was determined by liquid scintillation counting (see above).

Adenosine deaminase was determined by an ammonia liberation procedure as described previously [17]. Purine nucleoside phosphorylase and guanase activities were assayed by the methods of Stoeckler *et al.* [18] and Giusti [19] respectively. Assays for xanthine oxidase were performed essentially as described by Massey *et al.* [20], except that reactions were carried out at 30°. Methods for the determination of the activities of AMP kinase, GMP kinase, and nucleoside diphosphokinase were identical to those used by Agarwal *et al.* [21].

The activity of MTA phosphorylase was measured by a modification of the coupled spectrophotometric assay described by Pegg and Williams-Ashman [22]. Assays were carried out at 37°. Each cuvette contained 20 mM potassium phosphate buffer (pH 7.4), 0.8 units of xanthine oxidase, 500 μM 5′-deoxy-5′-methylthioadenosine, and appropriate amounts of the cell extracts (up to 400 μg protein) in a total volume of 1 ml. Since the presence of sulfhydryl reagents is required for maximal MTA phosphorylase activity [23], cell extracts were pretreated with dithiothreitol (1 mM final concentration) for at least 2 hr at 4° before assays were performed.

In most instances, enzyme activities were assayed on the same day as the extracts were prepared. In those cases where storage (24–48 hr at −20°) was necessary, enzyme activities determined after such storage were found to be essentially unchanged from those measured in fresh extracts. Enzyme activities are expressed as nmoles of substrate converted to product per min per mg protein. The method of Lowry *et al.* [24] was used to measure protein concentrations.

RESULTS

The activities of eleven enzymes of purine base, nucleoside, and nucleotide metabolism in cultured human colon carcinoma cells are presented in

* Abbreviations and enzyme nomenclature used: APRT, adenine phosphoribosyltransferase, EC 2.4.2.7; HPRT, hypoxanthine phosphoribosyltransferase, EC 2.4.2.8; adenosine kinase, EC 2.7.1.20; ADA, adenosine deaminase, EC 3.5.4.4; PNP, purine nucleoside phosphorylase, EC 2.4.2.1; MTA phosphorylase, 5′-deoxy-5′-methylthioadenosine phosphorylase; xanthine oxidase, EC 1.2.3.2; guanase, guanine deaminase, EC 3.5.4.3; AMP kinase, adenylate kinase, EC 2.7.4.3; GMP kinase, guanylate kinase, EC 2.7.4.8; and NDP kinase, nucleoside diphosphokinase, EC 2.7.4.6.

Table 1. Activities of purine-metabolizing enzymes in extracts of cultured human colon carcinoma cells*

Enzyme	DLD-1 Clone A†	DLD-1 Clone D†	HCT-15†	DLD-2‡
APRT	1.3 ± 0.2	1.6 ± 0.6	1.3 ± 0.3	1.8 ± 0.3
HPRT	1.2 ± 0.2	1.2 ± 0.5	1.2 ± 0.3	0.9 ± 0.1
Adenosine kinase	0.7 ± 0.2	0.4 ± 0.1	0.8 ± 0.2	0.4 ± 0.1
ADA	5.6 ± 0.5	1.7 ± 0.5	0.9 ± 0.1	2.4 ± 0.7
PNP	33 ± 6	36 ± 11	41 ± 11	123 ± 4
MTA phosphorylase	1.9 ± 0.2	1.9 ± 0.1	1.6 ± 0.2	1.4 ± 0.1
Guanase	13.6 ± 2.4	18.3 ± 4	3.7 ± 0.6	2.4 ± 0.5
Xanthine oxidase	—	—	—	—
AMP kinase	85 ± 18	91 ± 31	139 ± 43	122 ± 2
GMP kinase	7.9 ± 1.3	12.2 ± 2.3	7.5 ± 1.5	5.8 ± 0.2
NDP kinase	3380 ± 210	2608 ± 586	2529 ± 828	2548 ± 386

* Activities are expressed as nmoles of substrate converted to product per min per mg protein.
† Values given are means ± S.D. of three independent experiments.
‡ Values given are means ± S.D. of two independent experiments.

Table 1. With three exceptions, the marked similarity of each of these activities among the four distinct cell lines was apparent. Of the enzymes examined, ADA, PNP and guanase showed the most variation among the four lines. ADA activity differed more than 5-fold between HCT-15 and DLD-1 clone A cells. The value for ADA in DLD-2 cells was about 2-fold that for HCT-15 cells. The activity of PNP on the other hand, did not vary among clone A, clone D or HCT-15 cells, but these values were significantly lower than those determined for DLD-2 cells. Guanase activity in both clone A and D cells was 4- to 6-fold greater than that found in HCT-15 and DLD-2 cells. None of the activities of any of the other enzymes tested varied by more than 2-fold among the four cell lines. Xanthine oxidase activity was not detectable in any of the cell lines. The identification of MTA phosphorylase activity in these human colon carcinoma cells is the first reported occurrence of this enzyme in human neoplasms.

Data presented in Table 2 illustrates the activities of purine-metabolizing enzymes in human colon carcinoma xenografts in nude mice. As was the case with the *in vitro* study, in general, the activities of individual enzymes did not vary more than 2- to 3-fold among the four xenografts. The values for APRT activity in DLD-1 clone A and HCT-15 tumors were almost identical and were about one-half that for the clone D tumor. HPRT activity was very similar in clone A, clone D and HCT-15 tumors, but was 2-fold lower in DLD-2. ADA activity in DLD-2 tumors was more than 2-fold greater than the values for clone A and HCT-15 tumors. PNP activity was essentially identical for both clone A and HCT-15 tumors, but was elevated over this value by about 2- and 3-fold in clone D and DLD-2 tumors respectively. MTA phosphorylase activities were similar among the four tumors. Guanase activity was 2- to 3-fold lower in HCT-15 and DLD-2 tumors when compared to the values determined for clones A and D. As was the case with cells in culture, xanthine oxidase activity was not seen in any of the colon tumor xenografts. AMP kinase was lower in DLD-2 than in the other three tumors. The activity of GMP kinase, very similar in clone A, HCT-15 and DLD-2 tumors, was elevated more than 4-fold over this value in clone D tumors. Clone D tumors exhibited higher activities of NDP kinase than the other three tumors.

For comparison, Table 2 also lists the activities of these purine enzymes in normal human colon tissue. In many cases, the values obtained for normal tissue

Table 2. Activities of purine-metabolizing enzymes in extracts of human colon carcinoma xenografts and normal colon*

Enzyme	DLD-1 Clone A†	DLD-1 Clone D†	HCT-15†	DLD-2‡	Normal colon‡
APRT	1.4 ± 0.4	3.0 ± 0.3	1.7 ± 0.1	0.8 ± 0.1	0.8 ± 0.2
HPRT	0.9 ± 0.1	1.1 ± 0.2	1.0 ± 0.2	0.4 ± 0.1	0.3 ± 0.1
Adenosine kinase	0.3 ± 0.1	0.3 ± 0.2	0.4 ± 0.2	0.2 ± 0.1	0.2 ± 0.1
ADA	3.6 ± 1.5	7.5 ± 2.5	3.3 ± 1.2	11.3 ± 0.6	3.5 ± 0.9
PNP	38 ± 7	63 ± 3	31 ± 2	98 ± 6	35 ± 3
MTA phosphorylase	1.8 ± 0.2	2.3 ± 0.1	1.5 ± 0.2	1.4 ± 0.1	1.0 ± 0.1
Guanase	10.6 ± 2.0	9.6 ± 2.2	5.8 ± 0.5	3.5 ± 0.2	3.9 ± 2.1
Xanthine oxidase	—	—	—	—	—
AMP kinase	205 ± 3	271 ± 61	188 ± 15	128 ± 6	144 ± 6
GMP kinase	6.3 ± 0.6	27.6 ± 0.3	5.4 ± 0.1	5.1 ± 0.1	7.6 ± 1.7
NDP kinase	3050 ± 483	6223 ± 558	2457 ± 106	2000 ± 102	1660 ± 170

* Activities are expressed as nmoles of substrate converted to product per min per mg protein.
† Values given are means ± S.D. of three independent experiments.
‡ Values given are means ± S.D. of two independent experiments.

correlate quite well with those for the tumors. When normal tissue and clone A tumors were compared, the values for APRT, adenosine kinase, ADA, PNP, MTA phosphorylase, AMP kinase and GMP kinase were within 2-fold in each case. Interestingly, the activities of those enzymes that were different (HPRT, guanase and NDP kinase) were lower in the normal tissue. The values for APRT, HPRT, MTA phosphorylase, guanase, GMP kinase and NDP kinase were higher in clone D tumor than in normal tissue. In HCT-15 tumors, only HPRT activity was elevated over the value for normal tissue. The activities of both ADA and PNP were significantly lower in normal colon than in DLD-2 xenografts. From these data, it is apparent that the better-differentiated lines (HCT-15 and DLD-2) were more like normal colon in their enzyme profiles than were the more undifferentiated clones A and D.

When one examines the activities of the eleven purine-metabolizing enzymes assayed in this study in human colon carcinoma cells growing in monolayer culture (Table 1) and as xenografts in nude mice (Table 2), it is apparent that in most instances the activities did not change greatly from two-dimensional to three-dimensional tissue growth. Thus, for DLD-1 clone A, only the value for AMP kinase was changed significantly (2-fold higher in the xenograft). The activities of ADA and NDP kinase were elevated more than 2-fold in the xenograft clone D tumor as compared to clone D cells in culture. For HCT-15, only the value for ADA (elevated 2-fold in the xenograft) was changed. The values for APRT and HPRT were lower in the xenograft than in the cultured cells in the case of DLD-2, whereas that for ADA was elevated more than 3-fold in the xenograft. Even with the changes noted, out of a total of forty-four comparisons, significant (2-fold or greater) differences were seen in only seven instances.

DISCUSSION

It is of some interest to identify enzymes of human colon cancer cells that would provide suitable targets for chemotherapy. A biochemical target that is either absent from a number of patients' tumors, or is present in such variable amounts that no one drug protocol or dose can be used, is not exploitable. Therefore, in order for a drug to be effective against colon cancer in general, the target enzyme should be present in all, or at least most, colon tumors, and the activity of the enzyme should be relatively constant in these carcinomas. The data obtained in this study allow the enzymes assayed to be placed in two categories: one includes those whose individual activities remain fairly constant from one tumor line to the next, and the other includes those whose individual activities vary among the lines.

The great majority of the enzymes analyzed in this study show a fairly uniform activity among the four lines or their derivative tumors for any given enzyme. Since the cell lines differ in several properties (see Methods, "Cell lines"), the overall agreement among the four lines in the activity of any specific enzyme is noteworthy. Furthermore, if one considers that the four colon tumors we have studied represent the spectrum of histological differentiation available to this tumor type, the agreement is even more striking. This uniformity suggests that perhaps a number of enzymes in the purine metabolic pathway do not vary greatly in human colon cancer, and that any one of these enzymes might be available as a target in most human colon tumors. Of course, more colon carcinomas will have to be analyzed to determine, with certainty, if a set of enzymes with respective non-varying activities among tumors can be identified.

Two enzymes, ADA and guanase, showed individual variation among both the cell lines and their xenograft tumors. ADA activity varied from line to line with a maximum difference of about 5-fold between clone A and HCT-15 cells. Also, DLD-2 tumors had significantly higher ADA activity than two of the other three tumors. Therefore, the DLD-2 tumor might be more refractory than the other xenografts to treatment with an adenosine analog, if that analog is a substrate for ADA. Guanase activity was higher in the DLD-1 clones than in the other two parent lines and varied about 5-fold between clone D and DLD-2 cells. This suggests that clone A and D cells might be less sensitive to analogs such as 8-azaguanine (a substrate for guanase) than HCT-15 or DLD-2 cells would be.

Trotta and Balis [25] compared the activities of ADA in normal human colon and in human colon tumors removed surgically. Their data revealed greater ADA activity in the tumors [8.4 ± 1.4 nmoles $\cdot$ min^{-1} $\cdot$ (mg protein)$^{-1}$] than in the normal tissue [5.2 ± 1.1 nmoles $\cdot$ min^{-1} $\cdot$ (mg protein)$^{-1}$]. However, in only two of twelve patients were the differences in ADA activity between normal and tumor tissue greater than 2-fold (the criterion for significant difference used in our study). In our work, in only one tumor (DLD-2) were ADA levels different from those of normal tissue—activity in the tumor was higher. The ADA activity for normal human colon tissue reported by Trotta and Balis [25] is similar to that determined in our experiments [3.5 ± 0.9 nmoles $\cdot$ min^{-1} $\cdot$ (mg protein)$^{-1}$].

Xanthine oxidase activity was not detectable in any of the cultured cell lines, solid tumors or normal colon tissue. This observation is in agreement with that of Clynes *et al.* [26] who were unable to detect xanthine oxidase activity in several human tumor lines in culture. In contrast, the mucosa of the small intestine has been shown to contain this purine catabolic enzyme [27] and it has also been measured in human liver [27], kidney cortex [28], and hypernephromas [28].

When comparisons are made between the activities of purine-metabolizing enzymes in normal human colon tissue and in xenograft tumors, it is apparent that, where differences are observed, the activities in normal tissue are almost always lower than those for the xenografts. In the case of anabolic enzymes, this difference could be clinically advantageous. Thus, if an adenine analog were used as a chemotherapeutic agent, some selectivity might be obtained against tumors of the clone A, clone D or HCT-15 type as compared to normal tissue, because APRT activity is higher in the tumors than in normal colon. In the case of certain catabolic enzymes, higher activity in the tumor could be a disadvantage

or an advantage. The use of an adenosine analog that is a substrate for ADA might be limited in tumors of the DLD-2 type because of the higher activity of this enzyme in the tumor as compared to the normal tissue. Higher PNP activity in the tumor (clone D, DLD-2) as compared to normal tissue might confer selectivity on the tumor when guanosine or inosine analogs that are good substrates for PNP are used as chemotherapeutic agents.

Although *in vitro* testing of potential anticancer agents is a time-honored method of screening for compounds that may have chemotherapeutic potential, it often does not provide an accurate prediction of the *in vivo* effectiveness of a new drug [29]. One important reason for a discrepancy in results obtained from tissue culture cells compared to the same cells in a solid tumor would be a difference in the levels of a key enzyme in the two-dimensional monolayer versus the three-dimensional tumor. High levels of a target enzyme in cells growing in culture might favor a good *in vitro* response to the appropriate drug, whereas lower levels of this enzyme in tumors produced by inoculation of a suitable host with these same cells could indicate a less favourable *in vivo* response. For an *in vitro* test system to be predictive of *in vivo* efficacy, one requirement would be that the key enzymes in a pathway by which a particular drug would be metabolized should be present at about the same levels, both in the target cells in culture and in the solid tumor. This study shows that our spectrum of human colon cancer cell lines and their corresponding tumors in nude mice satisfy this requirement. Where variations are seen, they are small and probably not significant; *in vivo* and *in vitro* levels of an enzyme in a given cell type rarely differ by more than a factor of two. This represents an excellent conservation of enzyme activity in the transition of a cell from a culture monolayer to tissue architecture, and indicates our model is quite stable in this most important property. Although host metabolism, transport, etc. can be expected to complicate *in vivo* testing of purine analogs proven to be effective against the cultured colon carcinoma cells used in this study, the investigation should not be compromised because of significant differences in the levels of critical enzymes in target cells in culture compared to those in the same cell-type in a xenograft tumor.

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