Characterization of Folate Receptor from Normal and Neoplastic Murine Tissue: Influence of Dietary Folate on Folate Receptor Expression


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ABSTRACT

Membrane-associated folate receptors (FRs) have been detected in many mammalian species, and multiple isoforms have been identified. The pharmacological properties of FRs from murine kidney, liver, and six murine tumors were characterized. Murine kidney expressed primarily folate-binding protein 1, analogous to human FR-α, whereas murine liver expressed predominantly folate-binding protein 2, analogous to human FR-β. Five of six murine tumors expressed high-affinity FRs with pharmacological properties consistent with folate-binding protein 1 isoform expression. Restriction of dietary folate resulted in significant changes in the FR expression in most murine tissues. Kidney and tumor FRs showed a decreased affinity for folic acid, suggesting a change in isoform expression in response to a low folate diet. Density of the FR in the kidney decreased, and, in contrast, density of the FR in all tumors increased. The response of the liver to a low folate diet was unique in that there were no detectable changes in affinity or density of liver FR. Changes in dietary folate that modulate FR isoform expression may have relevance for cancer patients treated with antifolates.

INTRODUCTION

Folates are involved in essential one-carbon transfer reactions and play an integral role in cellular growth and development. FRs are glycosyl-phosphatidylinositol-linked proteins with high affinity for folic acid and 5-methyltetrahydrofolate. These proteins mediate the transport of physiological folates into the cytoplasm of cultured cells and have been implicated as the transporters of several novel antifolate compounds. However, the physiological role and significance of FRs in the acquisition and cellular metabolism of folate in vivo have not been clearly established. Furthermore, other transport mechanisms, such as the RFC, also play a significant role in folate accumulation. Potocytosis, a postulated mechanism of coordinated transport, is an active area of investigation.

Expression of FRs has been shown to be tissue specific. Three isoforms with high homology (FR-α, FR-β, and FR-γ) have been identified in human tissues and tumors. Ross et al. reported that mRNA coding for FR-α was expressed at high levels in tumors of epithelial derivation such as ovarian and colon carcinoma, whereas mRNA for FR-β was elevated more frequently in tumors of nonepithelial origin. Normal tissues expressed insignificant to low amounts of FR-α, whereas FR-β was more ubiquitously distributed albeit at low levels.

Cultured immortalized tumor cell lines preferentially expressed FR-α; this observation was not consistent with the FR expression patterns in vivo. These data suggest that cells may be pluripotent with respect to the FR isoform expression. Several other species express high-affinity FR with considerable homology to human FR. For example, murine leukemia L1210 cells express FBP1 and FBP2, which have significant homology to human FR-α and FR-β, respectively.

Regulation of FR expression is poorly understood. However, recent studies have begun to explore the role of dietary folate in the modulation of folate homeostasis. Studies by Schmitz et al. revealed significant changes in tissue folate pools in response to restriction of dietary folate. Also, Mendelsohn et al. reported that mice maintained on a LFD for 2 weeks had a significant increase in the folate-activating enzyme, folylypolyglutamate synthetase. These studies indicate that changes in nutritional folate produce variable yet significant effects on regulatory mechanisms involved in folate homeostasis. Additionally, in murine studies and early Phase 1 clinical trials, folic acid supplementation has been shown to modulate the efficacy and toxicity of antifolates such as lometrexol.

The potential importance of FRs as a cellular determinant of antifolate efficacy and toxicity and the influence of dietary folate on folate homeostasis led us to characterize FRs in murine tumors and tissues and to investigate the effect of dietary folate on receptor expression. Our results show that murine kidney expressed primarily FBP1, whereas murine liver expressed primarily FBP2. We present evidence that decreased dietary folate significantly alters the biochemical properties of murine kidney and tumor FRs and may result in increased expression of other isoforms of FRs in these tissues. The potential significance of alterations in the FR isoform expression with respect to antifolate therapy is discussed.
MATERIALS AND METHODS

Materials. Radiolabeled folic acid (\(^{125}\text{I}\)-labeled folic acid histamine derivative; 2200 Ci/mmol, NEX-114) was purchased from Dupont New England Nuclear Radiochemicals (Boston, MA). Dialyzed fetal bovine serum was obtained from Hyclone (Logan, UT). RPMI 1640 was purchased from Whittaker Bioproducts (Walkersville, MD). Bio-Rad protein assay dye reagent concentrate was purchased from Bio-Rad Laboratories (Melville, NY). FBP-148 Whatman GF/B filter paper was obtained from Biomedical Research and Development Laboratories, Inc. (Gaithersburg, MD). JMP software was purchased from SAS Institute, Inc. (Cary, NC).

Chemicals. Compounds LY237147, LY243246, lometrexol, LY254704, ZD-l694, LY254155, LY309886, and LY309887 were synthesized at Lilly Research Laboratories (Indianapolis, IN). LY237147 is a mixture of the 6R and 6S diastereomers of DDATHF (20). LY243246 is the 6S diastereomer of DDATHF, and lometrexol (LY249543) is the disodium salt of the 6R diastereomer of DDATHF. LY254704 is homo-DDATHF, in which a three-carbon chain between the tetrahydropyrido[2,3-d]-pyrimidine ring and the benzene ring replaces the two-carbon chain of DDATHF. ZD-1694 is a quinazoline inhibitor of thymidylate synthase (21). LY254155 is (6R,S)-2'5'-thienyl-DDATHF. LY309886 is the 6S diastereomer of this thiphene analogue, and LY309887 is the 6R diastereomer. All other chemicals were analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MO).

Murine Diets. Mice were maintained on one of the following diets for 2 weeks prior to tissue sampling: SD, Purina Chow 5001 containing 5.9 ppm folate; and LFD, Purina Chow 5831C-2, containing 0.032 ppm folate. Mice maintained on this LFD regimen have significantly decreased reduced folate pools (16). Specifically, tumor and liver tumors were implanted by trocar in the axillary region 10 days later. Tumor and liver tissues were obtained from murine tissues or tumors as described above. Briefly, tissues were excised from mice asphyxiated with CO,, placed in 10 ml ice-cold RPMI 1640, homogenized with a Tekmar homogenizer, and centrifuged at 800 \(\times\) g for 15 min to remove cellular debris. The supernatant was decanted, brought to 10 ml with 10 mM phosphate buffer (pH 7.4), and centrifuged at 43,500 \(\times\) g for 30 min. The remaining procedure was as described above for FDKB. Protein content was determined using the Bio-Rad microassay with bovine \(\gamma\)-globulin as a standard.

Receptor-binding Studies. Receptor assays were performed using membranes prepared from murine tissues or tumors as described above. Briefly, assays were performed in a final volume of 1.0 ml containing 10 mM phosphate buffer (pH 7.4). \(^{125}\text{I}\)-labeled folic acid derivative, unlabeled folic acid, or antifolate at the indicated concentrations, and membrane protein. The use of a high-specific activity ligand (22) enhanced the sensitivity of this assay, particularly in tissues which had lower receptor density. The following \(^{125}\text{I}\)-labeled folic acid derivative concentrations were used: 0.01 nM for KB cell, 0.02 nM for murine kidney, and 0.04 nM for murine liver and tumor. Binding was initiated by the addition of radiolabel, and the mixtures were allowed to come to equilibrium (2 h) at 25°C in the dark. Reaction solutions were rapidly filtered through GF/B filter papers presoaked in 0.1% polyethyleneimine using a Brandel Harvester (Gaithersburg, MD). Filter discs, rinsed three times with phosphate buffer, were counted for 1 min using a Compugamma model 1282 gamma counter (Wallac, Inc., Gaithersburg, MD). Nonspecific binding of label was determined by measuring bound radiolabeled folic acid in the presence of excess nonlabeled folic acid (30 nM). For each tissue studied, specific binding of labeled folate was measured over a range of protein concentrations (0–400 \(\mu\)g/ml) to determine the protein concentrations at which binding increased linearly. Membrane protein (0.5 \(\mu\)g KB cells, 20 \(\mu\)g kidney, 75 \(\mu\)g murine kidney, and 25–50 \(\mu\)g tumor) was incubated with \(^{125}\text{I}\)-labeled folic acid derivative at their respective concentrations in the presence of increasing concentrations up to 30 mM unlabeled folic acid. The amount of specifically bound folic acid at increasing concentrations of total folate was calculated using isotope dilution. Dissociation constants (\(K_d\)) and receptor densities (\(B_{max}\)) were calculated using JMP software, which determined the best nonlinear least square fit of the data to saturation isotherms for one or two site models as described by Wainscott et al. (23) and using Scatchard analysis.

Dissociation Constants of Antifolates. Antifolates were prepared as 10 mM stock solutions by dissolution in DMSO. Membrane protein (1 \(\mu\)g/ml KB cells, 30 \(\mu\)g/ml kidney, and 75 \(\mu\)g/ml murine liver) was incubated with \(^{125}\text{I}\)-labeled folic acid derivative (0.01–0.04 nM) and test compound (0.3–3000 nM) in a final volume of 0.5 ml. Dilutions and additions were per-
formed by a Beckman Biomek 1000 Automated Laboratory Workstation (Fullerton, CA). Concentrations yielding 50% displacement of labeled folate (IC_{50}) were determined by nonlinearly fitting the data to a two-parameter logistic model using JMP software. Dissociation constants (K_d) were generated using the Cheng-Prusoff equation (24), with IC_{50} equal to the concentration of competitor which decreased folic acid binding by 50%. L equal to the concentration of 125I-labeled folic acid derivative, and K_d equal to the equilibrium dissociation constant for folic acid.

**Statistical Analysis.** Student’s t test was utilized to obtain estimates of statistical significance of the data. Any P < 0.05 was considered significant.

**PCR Analysis of Human FR-α and FR-β and Murine FBPI and FBP2.** Total RNA was isolated from human KB epidermoid carcinoma cells and from the livers of female BALB/c mice maintained on a SD using RNAzol B (Biotex Laboratories, Houston, TX). Poly(A)^+ RNA was isolated from total liver RNA using a prepacked oligo(dT)-cellulose type 3 column (Collaborative Biomedical Products, Bedford, MA). cDNA was synthesized from 1 μg total KB RNA and 1 μg poly(A)^+ BALB/c liver RNA using the SuperScript Premplification System (Life Technologies, Inc., Gaithersburg, MD). Mouse kidney cDNA (7135–1) synthesized from poly(A)^+ RNA of adult male BALB/c mice was purchased from Clontech (Palo Alto, CA). PCRs were performed utilizing GeneAmp PCR Core Reagents (N808–0009) from Perkin Elmer/Cetus (Branchburg, NJ). Two μl of the above cDNA reactions and 1 μl Clontech Quick-Clone mouse kidney cDNA were amplified in 100 μl containing 1.5 mm MgCl2, 5 units AmpliTaq polymerase, and 20 pmol of the appropriate primers. Human FR-α and FR-β sequences were amplified using primers described by Shen et al. (10). Primers for amplification of murine FBPI and FBP2 were designed using the published sequences from Brigle et al. (446 bp; Ref. 25) and human cathepsin D (1238 bp; Ref. 26) as well as from human FDKB cells. The results of RT-PCR were amplified as controls for cDNA integrity. All primers were prepared by Bruce Glover at Eli Lilly. cDNA was amplified 35 cycles, each cycle consisting of 1 min at 95°C for denaturation, 2 min at 50°C for annealing, and 4 min at 72°C for polymerization. PCR products were visualized with ethidium bromide after electrophoresis through a 4% agarose gel. Molecular weight markers V and VII were purchased from Boehringer Mannheim (Indianapolis, IN).

**RESULTS**

**FRs in Normal Murine Tissues.** The affinity for folic acid and density of FRs in murine kidney and liver were determined (Table 1). Kidney expressed FRs with high affinity for folic acid; FRs from mice on a SD had a K_d of 0.047 nm, whereas receptors from mice on LFD had a K_d of 0.139 nm (P < 0.0002). Overall, murine kidney expressed high levels of FRs. However, restriction of dietary folate resulted in a significant decrease in kidney receptor density from 884 fmol/mg protein to 504 fmol/mg protein (P < 0.001). Therefore, in the kidney, dietary folate had significant effects on both the affinity of FRs for folic acid as well as the density of FRs. Livers expressed FRs with significantly lower affinity for folic acid than kidney FRs (K_d > 0.5 nm, P < 0.04), and, in contrast to the kidney, dietary folate did not have an effect on receptor affinity or density.

**Table 1** Dissociation binding constants and receptor densities of FR in murine kidney and liver

<table>
<thead>
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<th>SD</th>
<th>LFD</th>
<th>SD</th>
<th>LFD</th>
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<tbody>
<tr>
<td>K_d (nm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>0.047 ± 0.008</td>
<td>0.139 ± 0.011</td>
<td>884 ± 54</td>
<td>504 ± 42*</td>
</tr>
<tr>
<td>Liver</td>
<td>0.542 ± 0.084</td>
<td>0.501 ± 0.114</td>
<td>72 ± 10</td>
<td>78 ± 8</td>
</tr>
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</table>

* Statistically significant difference from the corresponding SD value.

**Binding Characteristics of FRs in Murine Kidney and Liver and FDKB Cells.** The 11-fold difference between kidney and liver FR dissociation constants for folic acid suggested that these receptors may represent different isoforms of the FR. To more fully explore this hypothesis, a series of competitive inhibition studies were conducted to assess the affinities of these receptors, as well as human FDKB cell FR, for folic acid and nine antifolates (Table 2). FDKB cells expressed FRs with a high affinity (K_d < 2.2 nm) for most compounds tested, with the exceptions of methotrexate and ZD-1694, both of which are transported primarily through the RFC (6). Murine kidney receptor bound all compounds with a similar affinity to the human FDKB receptor (r^2 = 0.99). In contrast, the murine liver FR had a 5–84-fold lower affinity for all compounds, with the exception of LY254704 (homo-DDATFH) which was bound tightly by all receptor types. These data illustrate the significant difference in binding properties of kidney and liver FRs and suggest the presence of different FR isoforms in these tissues.

**Expression of FR Isoforms in Murine Kidney and Liver and FDKB Cells.** RT-PCR was used to clarify the relationship between murine kidney and liver FRs from mice on a SD as well as from human FDKB cells. The results of RT-PCR using specific primers for murine FBPI and FBP2 and human FR-α and FR-β (Fig. 1) showed that the message for FR-α was expressed by FDKB cells, and the message for FBPI, the murine homologue of FR-α, was detected in murine kidney. Murine FBP2 was only detected in murine liver. These data are consistent with the observed differences in binding affinities of antifolates between kidney and liver FRs and suggest that these tissues express FBPI and FBP2 isoforms of FRs, respectively.

**FRs in Neoplastic Murine Tissues.** Tumors from mice on a SD expressed FRs with high affinities for folic acid; dissociation constants ranged from 0.046 nm for Lewis lung carcinoma to 0.117 nm for C6 colon carcinoma (Table 3). X5563 myeloma had no detectable FRs when maintained in SD mice. Restriction of dietary folate resulted in significant changes in the dissociation constants of the tumor FRs; FRs from tumors of...
mice on a LFD had a 2–5-fold decrease in affinity for folic acid. This decrease in affinity was significant in all tumors tested ($P < 0.04$), with the exception of C6 colon carcinoma, which demonstrated the same trend without reaching statistical significance ($P < 0.33$). The density of FRs in tumors varied considerably. However, all tumors demonstrated a trend of increasing receptor density (1.4–2.3-fold) in response to restricted dietary folate. In particular, FRs, undetectable using up to 400 µg X563 myeloma membrane protein from SD mice, were readily detectable using 100 µg X563 myeloma membrane protein from LFD mice (32 fmol/mg protein). These results are in contrast to those obtained from the kidney, where a statistically significant decrease in receptor density was observed in response to restricted dietary folate, and in liver where a LFD had no effect on receptor density.

**Receptor Analysis of M5076 RCOS.** The observance of lower affinity FRs in tumors in response to a LFD suggested that a second FR isoform might be expressed under conditions of restricted dietary folate. This phenomenon was investigated utilizing M5076 RCOS from mice maintained on a LFD. Expanded competitive displacement assays using 20 folic acid concentrations over a range of 0–30 nM were conducted. Previous studies utilizing M5076 RCOS from mice on a SD demonstrated the presence of a single high-affinity FR with a $K_d$ of 0.068 nM and $B_{max}$ of 169 fmol/mg protein ($n = 3$; Fig. 2A). In contrast, two of four experiments utilizing M5076 RCOS from LFD mice resulted in curvilinear Scatchard plots (Fig. 2B). Resolution of these binding sites yielded a high-affinity receptor ($K_d = 0.055$ nM and $B_{max1} = 43$ fmol/mg protein) with properties similar to FBP1 and a second lower affinity site ($K_d = 2.0$ nM and $B_{max2} = 285$ fmol/mg protein). In the other two experiments, a single receptor of intermediate affinity ($K_d = 0.26$ nM) resulted from the Scatchard analysis (data not shown). These results suggest a heterogeneous response to a LFD in these tumors. The origin of this variability may relate to inherent differences in the tumor seed implanted or clonal selection during tumor outgrowth.

**DISCUSSION**

The role of FRs in folate homeostasis is poorly understood. Controversy exists regarding their importance for providing adequate folate for cells and their overexpression in certain human tumors. A clearer understanding of factors which modulate FR expression may provide insight to their role in normal and tumor folate physiology. Recent evidence has shown that changes in dietary folate produce variable yet significant effects on cellular parameters that affect antifolate efficacy and toxicity (16–18, 27). In addition, clinical experiences with antifolates such as methotrexate (28), 5-fluorouracil (29), and lomotrexol (19) have demonstrated that supplemental folates can significantly modulate efficacy and toxicity. Therefore, the possible role that nutritional folate plays in the modulation of FR expression needs to be considered. For these reasons, we characterized FRs in murine tissues and tumors and determined the effect of reduced dietary folate on FR expression.

Receptor characterization of tissues and tumors obtained from mice on a SD indicated that isoform expression was tissue specific. Murine kidney expressed a single high-affinity FR at high density. The pharmacological profile of this receptor was similar to that of the FR-$\alpha$ of human KB cells; PCR analysis confirmed that the isoform in kidney was murine FBP1 (analogous to human FR-$\alpha$). Liver expressed a single low-affinity FR with a significantly different pharmacological profile from kidney and human FDKB receptors. PCR analysis identified the presence of only murine FBP2 (analogous to human FR-$\beta$) in liver. Four of five tumors from mice on a SD expressed FRs with an affinity for folic acid similar to that of FRs from SD kidney. This suggested that C3H mammary, M5076 RCOS, B16 melanoma, and Lewis lung carcinomas expressed receptors consistent with the FBPI isoform. The C6 colon carcinoma had a slightly lower affinity for folic acid, suggesting that this tumor expressed a mixture of isoforms.

Changes in dietary folate had significant effects on murine kidney and tumor FR expression. In kidney, restriction of dietary folate resulted in significant decreases in both receptor affinity and density. Kidney FRs from mice on a LFD had nearly a 3-fold lower affinity for folic acid than FRs from mice on a SD, suggesting a shift in isoform expression. The density of FRs in kidney decreased by 42% in response to a LFD. This result was unexpected for several reasons. First, based on the observation that FR expression is up-regulated in cultured cells by decreased media folate (30–32), we hypothesized that a LFD would induce a compensatory increase in expression in murine tissues in vivo. These data suggest that cultured cells respond differently to low folate stress than tissues in vivo. Second, the mechanisms of renal transport and conservation of folates would lead one to expect an up-regulation of FRs under low folate conditions. McMartin et al. (33) found that during folate deficiency, urinary excretion of folate decreased as a conservation
Dissociation binding constants and receptor densities of FR in murine solid tumors

Saturation binding assays were conducted by incubating membrane protein from tumor obtained from mice on a SD or a LFD with a range of folic acid concentrations for 2 h at 25°C. Scatchard analysis yielded the affinity ($K_d$) and density ($B_{max}$) of the FR. Values are the mean of three to seven separate experimental determinations.

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>SD</th>
<th>LFD</th>
<th>SD</th>
<th>LFD</th>
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<tr>
<td>C3H mammary</td>
<td>0.078 ± 0.018</td>
<td>0.288 ± 0.048&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81 ± 16</td>
<td>185 ± 28&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>M5076 RCOS</td>
<td>0.068 ± 0.014</td>
<td>0.307 ± 0.032&lt;sup&gt;a&lt;/sup&gt;</td>
<td>169 ± 38</td>
<td>242 ± 22</td>
</tr>
<tr>
<td>B16 melanoma</td>
<td>0.053 ± 0.023</td>
<td>0.154 ± 0.023&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20 ± 5</td>
<td>45 ± 16</td>
</tr>
<tr>
<td>Lewis lung</td>
<td>0.046 ± 0.012</td>
<td>0.212 ± 0.028&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98 ± 27</td>
<td>187 ± 21</td>
</tr>
<tr>
<td>C6 colon</td>
<td>0.117 ± 0.046</td>
<td>0.190 ± 0.048</td>
<td>72 ± 10</td>
<td>160 ± 60</td>
</tr>
<tr>
<td>X5563 myeloma</td>
<td>Undetectable</td>
<td>0.281 ± 0.044</td>
<td>Undetectable</td>
<td>32 ± 3</td>
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</table>

<sup>a</sup> Statistically significant difference from the corresponding SD value.

Measure, suggesting renal adaptation to conserve folates through increased reabsorption by the kidney. Therefore, decreased receptor expression in response to a LFD seemed counterintuitive to the role of kidney in folate homeostasis. However, other investigators have observed similar phenomena in rat kidney. Rothenberg et al. observed a decrease in rat kidney FR and FR mRNA in response to a LFD. It appears, therefore, that levels of FRs in kidney do not reflect the efficiency of folate reabsorption.

Restriction of dietary folate affected the affinity of FRs for folic acid and the receptor density in murine tumors. A significant increase in dissociation constants was detected in four of five tumors from LFD mice compared to those of SD mice, suggesting that folate deprivation induced a change in FR isoform expression. In the case of M5076 RCOS, the higher dissociation constant was sometimes indicative of two distinct receptor sites, as determined by one- and two-receptor-site model analysis. The lower affinity receptor, expressed in 6-fold excess over the higher affinity site, became the predominant isoform in the LFD tumor. This shift in isoform expression to a receptor of lower affinity would seemingly compromise the ability of a tumor cell to obtain folate necessary for cell growth and proliferation; however, the decreased affinity may be offset by increased receptor density. Similar differentials in binding affinities of FRs from tumors of mice on a SD and a LFD were detected, suggesting that FBP1 was present in tumors from folate-replete animals, whereas restriction of dietary folate induced expression of a second FR isoform, possibly FBP2, concomitant with down-regulation of FBP1. Ross et al. (12) sampled adult human tumors using quantitative RT-PCR and

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<sup>a</sup> Personal communication.
found that all carcinomas expressed low levels of FR-β and significantly greater levels of FR-α, particularly in ovarian, breast, and lung carcinomas. These data suggest that tumors have the capacity to express multiple isoforms of the FRs.

The effect of a LFD on liver FRs was investigated because of the integral role of liver in folate storage and homeostasis. In contrast to findings with kidney, nutritional folate did not influence FR expression; only FBP2 was expressed at modest levels. da Costa and Rothenberg (34) also observed no effect of low dietary folate on FR levels in rat livers. Thus in liver, factors other than dietary folate on FR levels in rat livers. Thus in liver, factors other than dietary FR expression; only FBP2 was expressed at modest levels.

Cancer patients are often folate deficient as a result of their disease (36, 37). Saleh et al. (38) demonstrated that patients with metastatic disease incorporated more folic acid into their reduced folate pools, had decreased catabolism of folate, and had more rapid clearance of serum folate than controls, even in the presence of maintained serum 5-methyltetrahydrofolate concentrations. For these reasons, the effect of low dietary folate on biochemical modulators of folate bioavailability was evaluated. In the experiments reported here, a 2-week LFD regimen produced significant changes in FR affinities and densities in murine kidney and several murine solid tumors. The implications in the clinical setting could be profound, depending on whether the expressed FR is functional and physiologically significant. Our results suggest that the nutritional state of cancer patients may significantly modulate sensitivity to antifolates that utilize the FR. Most folates and antifolates have a 5–10-fold higher affinity for FR-α than FR-β, whereas some folate-based compounds have even higher selectivity (11). Therefore, conversion of the tumor FR isofrom from α to β may result in loss of selectivity of antifolates for tumor over normal tissue. Under folate-poor conditions, the differential affinity between tumor and liver FRs may narrow, resulting in enhanced uptake and storage of an antifolate in the liver (19). The cumulative and delayed toxicities observed with lometrexol (39) may be an example of this phenomenon. Supplementation of cancer patients with folinic acid might maintain the differential expression of FR isoforms and thereby preserve the tissue selectivity of antifolates. Utilization of antifolates that maximize the advantage conferred by this selectivity may enhance the development of antifolates as effective chemotherapeutic agents devoid of serious delayed toxicity.

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