Folic acid inhibition of EGFR-mediated proliferation in human colon cancer cell lines

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1 J ohn D. Dingell Veterans Affairs Medical Center and Departments of 2Internal Medicine, 3Pathology, and 4Biochemistry and Molecular Biology, and 5Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, Michigan 48201

Jaszewski, Richard, Ahmed Khan, Fazlul H. Sarkar, Omer Kucuk, Martin Tobi, Abbas Zagnoon, Ravi Dhar, Joseph Kinzie, and Adhip P. N. Majumdar. Folic acid inhibition of EGFR-mediated proliferation in human colon cancer cell lines. Am. J. Physiol. Cell Physiol. 277 (Cell Physiol. C1142–C1148, 1999.—Although accumulating evidence suggests a chemopreventive role for folic acid in colon cancer, the regulation of this process in unknown. We hypothesize that supplemental folic acid exerts its chemopreventive role by inhibiting mucosal hyperproliferation, an event considered to be central to the initiation of carcinogenesis in the gastrointestinal tract. The present investigation examines the effect of supplemental folic acid on proliferation of Caco-2 and HCT-116 colon cancer cell lines. Furthermore, because certain tyrosine kinases, particularly epidermal growth factor receptor (EGFR), play a role in regulating cell proliferation, we also examined the folic acid-induced changes in tyrosine kinase activity and expression of EGFR. In Caco-2 and HCT-116 cells, maintained in RPMI 1640 medium containing 1 µg/ml folic acid, we observed that the supplemental folic acid inhibited proliferation in a dose-dependent manner. Pretreatment of HCT-116 and Caco-2 cell lines with supplemental folic acid (1.25 µg/ml) completely abrogated transformed growth factor-α (TGF-α)-induced proliferation in both cell lines. Tyrosine kinase activity and the relative concentration of EGFR were markedly diminished in both cell lines following a 24-h exposure to supplemental folic acid. The folic acid-induced inhibition of EGFR tyrosine kinase activity in colon cancer cell lines was also associated with a concomitant reduction in the relative concentration of the 14-kDa membrane-bound precursor form of TGF-α. In conclusion, our data suggest that supplemental folic acid is effective in reducing proliferation in two unrelated colon cancer cell lines and that EGFR tyrosine kinase appears to be involved in regulating this process.

chemoprevention; epidermal growth factor receptor tyrosine kinase; transforming growth factor-α

ALTHOUGH THE EPIDEMIOLOGY of colorectal cancer is related to genetic susceptibility, dietary factors such as vitamins and micronutrients are thought to influence tumorigenic processes, which include proliferation, differentiation, and apoptosis (13, 46). There is increasing evidence to suggest that the water-soluble vitamin folic acid may have a chemopreventive role in colon tumorigenesis. Diets deficient in folic acid have been associated with a higher incidence of adenomas, the most frequent premalignant colorectal lesion (4, 13, 37). Folate deficiency also enhances carcinogen-induced colonic neoplasia in rodents (10). In addition, several large case-controlled studies have noted an inverse relationship between dietary folic acid and the development of colorectal cancer (5, 12, 21, 34). Furthermore, in ulcerative colitis, red blood cell folate levels are greatly reduced and inadequate dietary supplementation of folic acid results in the development of colonic dysplasia or cancer, whereas supplementation of the vitamin may be chemopreventive (21, 23, 41).

Although the regulatory mechanisms for folic acid-induced suppression of colorectal neoplasia are poorly understood, we hypothesize that supplemental folic acid may inhibit mucosal cell proliferation, an event thought to be central to the initiation of carcinogenesis in the gastrointestinal tract (25). In support of this, we have observed that stimulation of ornithine decarboxylase (ODC, the rate-limiting enzyme in polyamine biosynthesis pathway) and tyrosine kinase activities in rat colonic mucosal explants in response to methylazoxymethanol (the active metabolite of the colonic carcinogen azoxymethane) is greatly attenuated by supplemental folic acid (35). Although neither ODC nor tyrosine kinases are directly involved in cell proliferation, they show a positive relationship with cell proliferation (26, 29, 36). Moreover, the activity of these enzymes is also increased in conditions predisposed to dysplasia and neoplasia (2). However, to the best of our knowledge, no information is available as to whether supplemental folic acid will inhibit proliferation of colon cancer cells. Therefore, the primary objectives of this investigation were to determine the effect of supplemental folic acid on proliferation of colon cancer cell lines Caco-2 and HCT-116 and the mechanisms that regulate this process.

Accumulating evidence suggests that tyrosine kinases, which are associated with a number of growth factor receptors and products of many protooncogenes, play a critical role in regulating proliferation of normal, preneoplastic, and neoplastic cells (16, 47). With respect to the development and progression of colonic neoplasia, several lines of evidence suggest a role for epidermal growth factor receptor (EGFR) in regulating these processes. For example, colonic neoplasia is shown to be associated with overexpression of EGFR (3, 19). In addition, we have observed an increased tyrosine kinase activity of EGFR in the colonic mucosa of patients with ulcerative colitis, adenomatous polyps, and colon

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cancer (29). Moreover, in rats, administration of azoxy-
methane, which augments colonic mucosal prolifer-
tive activity, also stimulates EGFR tyrosine kinase
activity, and both processes can be attenuated by
tyroptin (28, 40), an inhibitor of tyrosine kinases
with a greater specificity for EGFR than other protein
kinases (24). It has also been demonstrated that mono-
clonal antibody to EGFR inhibits proliferation in cer-
tain colon cancer cell lines (18). Together, these results
suggest a role for EGFR in the development and
progression of colonic neoplasia. To determine, there-
fore, whether EGFR may also be involved in regulat-
ing the folic acid-induced inhibition of proliferation of colon
cancer cells, tyrosine kinase activity of EGFR was
measured in Caco-2 and HCT-116 cells following expo-
sure to this vitamin. Furthermore, because ligand-
induced activation of EGFR is one of the primary
causes of induction of its intrinsic tyrosine kinase (26),
we have also evaluated the role of endogenous trans-
forming growth factor-α (TGF-α) in regulating the
enzyme activity in colon cancer cells.

MATERIALS AND METHODS

Cell culture and cell proliferation. Caco-2 and HCT-116
cells were obtained from the American Type Culture Collec-
tion (Manassas, VA). Cells were maintained in RPMI 1640
supplemented with 10% fetal bovine serum (FBS), penicillin
(10,000 U/ml), streptomycin (10,000 U/ml), and amphotericin
(25 µg/ml) at 37°C in an atmosphere of 95% air and 5% CO2.

For determination of folic acid-induced changes in prolifera-
tion, 1-ml aliquots of cell suspension containing 7.5 × 10⁴
cells in RPMI 1640–10% FBS were plated in 24-well culture
dishes. At ~50% confluency, which occurs ~24 h after plating,
cells were incubated in the absence (control) or presence of
folic acid. In some experiments, aliquots of both cell lines,
maintained in RPMI 1640 containing 1 µg/ml folic acid and
10% FBS, were preincubated in the absence (control) or
presence of supplemental folic acid (1.25 µg/ml) for 48 h. After
preincubation, the control and folic acid-preincubated cells
were serum starved (0.1% FBS) for 24 h and then exposed to
TGF-α (10⁻⁸ M) for 24 h. In all experiments, cells were harvested with trypsin-EDTA solution and counted using a
hemocytometer.

EGFR tyrosine kinase activity. The enzyme activity was
determined as described previously (26). Briefly, cells were
homogenized in RIPA buffer [20 mM sodium phosphate, pH
7.4, containing 5 mM sodium pyrophosphate, 1% Triton
X-100, 0.1% SDS, 0.5% sodium deoxycholate, 150 mM NaCl,
5 mM EDTA, 5 mM phenylmethylsulfonyl fluoride (PMSF),
10 µg/ml leupeptin, 1 mM Na₃VO₄, 1 µg/ml aprotonin, and 1
mM 1,10-phenanthroline]. The homogenate was stirred for 30
min at 4°C and subsequently centrifuged at 10,000 g for 10
min. The supernatant, after it was diluted with an equal
volume of homogenizing buffer (10 mM HEPES, pH 7.2,
containing 150 mM NaCl, 1 mM MgCl₂, 1 mM PMSF, 1 mM
Na₃VO₄, 10 µg/ml leupeptin, and 1 mM 1,10-phenanthroline),
was used as the source for the enzyme. In all immunoprecipi-
tation studies, protein concentrations were standardized
among the samples. Protein content in all samples was
measured using the biocinchoninic acid protein assay kit from
Pierce (Rockford, IL) according to the manufacturer’s instruc-
tion.

For determination of EGFR tyrosine kinase activity, ali-
quotsof cell lysate containing 350 µg protein were incubated
overnight at 4°C with 1 µg polyclonal antibody to EGFR (UBI,
Lake Placid, NY). The immune complexes were precipitated
with Sepharose-bound protein G (Sigma-Aldrich, Steinheim,
Germany), washed several times with 1:1 RIPA-homogeniz-
ing buffer, and finally resuspended in 25 µl of assay buffer
(100 mM Hepes, pH 7.5, 10 mM MgCl₂, 80 mM KCl, and
4 mM 2-mercaptoethanol). The reaction at 30°C for 10
min was initiated by adding 20 µl of the reaction mix [2 µl
of acid-denatured enolase (enolase was denatured by adding
an equal volume of 0.1 M acetic acid and incubating the mixture
for 8 min at 30°C), 5 µl of 20 µM ATP, 5 µl of [γ³²P]ATP, and
13 µl of dH₂O for each sample]. The reaction was terminated
by adding 40 µl of gel loading buffer (62.5 mM Tris-HCl, pH
6.5, 6% SDS, 20% glycerol, 10% 2-mercaptoethanol, and 0.1
mM bromophenol blue) and subsequently subjected to SDS-PAGE
(27). After electrophoresis, the gel was fixed, washed, dried,
and finally exposed to X-Omat AR film. The extent of enolase
phosphorylation, as the measure of EGFR tyrosine kinase
activity, was analyzed by a PhosphorImager (Molecular Dy-
namics Strom 860, Sunnyvale, CA).

Western blot analysis of EGFR and TGF-α. EGFR was
immunoprecipitated from detergent-solubilized cells contain-
ing 1 mg protein with EGFR antibodies as stated above. In the
case of TGF-α, cells were homogenized in homogenizing
buffer and the homogenate was centrifuged at 30,000 g at 4°C
for 30 min to obtain the crude membrane fraction. The
membrane fraction was suspended in RIPA buffer. Aliquots of
the membrane fraction containing 3 mg of protein were
immunoprecipitated with monoclonal antibodies against
TGF-α (Santa Cruz Biotechnology, Santa Cruz, CA). EGFR
and TGF-α immunoprecipitates were subjected to 7.5% and
15% SDS-PAGE, respectively (27). After electrophoresis, pro-
tens were transferred electrophoretically onto polyvinyli-
dene difluoride membrane (Millipore, Bedford, MA) using a
transfer cell kit (International, Mount Prospect, IL). Mem-
branes were incubated overnight in 5% nonfat dry milk
(Sanalac) and 0.1% Tween 20 in 1× PBS. After membranes
were washed three times with 1× PBS and 0.1% Tween 20,
they were incubated at room temperature for 1 h with
respective antibodies at a final concentration between 1:2,500
and 1:1,000 in PBS containing 0.25% nonfat dry milk (San-
alac)-0.1% Tween 20. The membranes were washed and
subsequently incubated with horseradish peroxidase linked
to anti-mouse/anti-rabbit antibody conjugates (ICN) in PBS
containing 5% nonfat dry milk and 0.1% Tween 20. After
washing, the protein bands were visualized by an enhanced
chemiluminescence detection system (Amersham, Piscata-
way, NJ). The membranes containing the electrophoresed
proteins were exposed to X-Omat film, and the density of the
protein bands were analyzed by a digital image analysis
system (Molecular Dynamics Strom 860).

Statistical analysis. When applicable, results were statisti-
cally evaluated with Student’s t-test for unpaired values, with
P < 0.05 designated as the level of significance.

RESULTS

In the present investigation, all experiments were
performed using two colon cancer cell lines, Caco-2 and
HCT-116. Reasons for using these two colon cancer cell
lines were to determine whether supplemental folic
acid will be effective in inhibiting proliferation in both
colon cancer cell lines. Although both cell lines are
derived from colonic carcinomas, there are significant
morphological and phenotypic differences between them.
For example, Caco-2 cells lose their proliferative capac-
ity on reaching confluence, resulting in a marked rise in
activities of sucrase-isomaltase and other brush-border
enzymes, which are considered equivalent to the crypt- to-villus differentiation occurring in vivo (14, 39). However, these changes are not apparent in HCT-116 cells, which are less differentiated compared with Caco-2 cells, and, unlike Caco-2 cells, they have lost the ability to spontaneously differentiate (7).

In the first set of experiments, Caco-2 and HCT-116 cells, which were maintained in RPMI 1640 containing 1 µg/ml folic acid, were further exposed to increasing concentrations (0–3.1 µg/ml) of folic acid for 48 h. In both cell lines, supplemental folic acid inhibited proliferation in a dose-dependent manner (Fig. 1).

To further determine the antiproliferative effect of folic acid on colon cancer cell lines, the next set of experiments was performed to examine whether pretreatment of HCT-116 and Caco-2 cells for 48 h with folic acid arrests TGF-α-induced proliferation of HCT-116 and Caco-2 cells. Aliquots of both cell lines were preincubated in the absence or presence of folic acid (1.25 µg/ml), subsequently serum starved for 24 h, and then exposed to TGF-α (10^{-10} M) for another 24 h. Preincubation with folic acid caused a 40–50% reduction in proliferation when compared with the control (Fig. 1).

Preincubation with folic acid for 24 h resulted in a significant 70–80% increase in proliferation over the corresponding basal levels (Fig. 2). However, pretreatment with folic acid completely abrogated the stimulatory effect of the growth factor by reducing proliferation by 59% and 81% in Caco-2 and HCT-116 cells, respectively, when compared with the corresponding basal levels (Fig. 2).

Although the regulatory mechanism(s) for folic acid-induced inhibition of proliferation of colon cancer cell lines is unknown, we hypothesized that certain tyrosine kinases, specifically the enzyme associated with EGFR, may play a key role in this process. To test this hypothesis, we studied the effect of supplemental folic acid on EGFR tyrosine kinase activity in HCT-116 and Caco-2 cells. Exposure of Caco-2 and HCT-116 cells (those not preincubated with folic acid) to 10^{-10} M TGF-α for 24 h resulted in a significantly reduced 70–80% decrease in EGFR expression compared with the respective basal levels (Fig. 3).

To determine whether the suppressive effect of folic acid on EGFR tyrosine kinase activity may be related to decreased levels of the enzyme, Western blot analysis of the membrane fraction was performed to determine changes in the relative concentration of EGFR in both Caco-2 and HCT-116 cells following exposure to a supplemental dose of folic acid (0.625 µg/ml) for 24 h. Folic acid caused a 75% and 80% reduction in EGFR expression in HCT-116 and Caco-2 cells, respectively, when compared with the corresponding basal levels (Fig. 4).

Because ligand binding to the extracellular domain of EGFR is one of the primary causes for activation of the receptor's intrinsic tyrosine kinase activity (38), any event that affects the ligand-receptor interaction is likely to have a profound effect on EGFR activation. TGF-α, one of the primary ligands of EGFR, is a membrane-bound peptide that also activates EGFR (6, 30, 36). To determine whether folic acid-induced inhibition of EGFR tyrosine kinase activity in colon cancer cell lines may be related to parallel changes in membrane-bound TGF-α, we examined the effect of this vitamin on membrane expression of TGF-α in both cell lines. Western blot analysis of the membrane fraction revealed several molecular forms of TGF-α with relative molecular masses of between 14 and 18 kDa in HCT-116 and Caco-2 cells, whereas those exposed to folic acid (0.625 µg/ml) for 24 h showed only one prominent molecular form of the peptide with a relative molecular mass of 14 kDa in these cells (Fig. 5). The relative concentration of the 14-kDa TGF-α in folic acid-treated cells was found to be substantially lower compared with the corresponding band from control cells (Fig. 5).

The more profound beneficial effect of supplemental folic acid relative to decreased levels of the enzyme, Western blot analysis of the membrane fraction was performed to determine changes in the relative concentration of EGFR in both Caco-2 and HCT-116 cells following exposure to a supplemental dose of folic acid (0.625 µg/ml) for 24 h. Folic acid caused a 75% and 80% reduction in EGFR expression in HCT-116 and Caco-2 cells, respectively, when compared with the corresponding basal levels (Fig. 3).

Although the number of studies performed is relatively small, murine and human studies suggest a role for folic acid in reducing colon carcinogenesis (13, 36). It has been demonstrated that a diet deficient in folic acid may be associated with an increased risk of colorectal neoplasia (21, 37), whereas dietary supplementation of this nutrient may be chemopreventive (22, 23). Several case-control studies have noted an inverse relationship between folic acid intake and the risk for developing colorectal cancer (5, 12, 13, 21, 34).

DISCUSSION

There is an accumulating body of evidence suggesting that vitamins and micronutrients may have a key role in reducing the susceptibility to colorectal cancer (13, 46). It has been demonstrated that a diet deficient in folic acid may be associated with an increased risk of colorectal neoplasia (21, 37), whereas dietary supplementation of this nutrient may be chemopreventive (22, 23). Several case-control studies have noted an inverse relationship between folic acid intake and the risk for developing colorectal cancer (5, 12, 13, 21, 34).

The more profound beneficial effect of supplemental folic acid relative to decreased levels of the enzyme, Western blot analysis of the membrane fraction was performed to determine changes in the relative concentration of EGFR in both Caco-2 and HCT-116 cells following exposure to a supplemental dose of folic acid (0.625 µg/ml) for 24 h. Folic acid caused a 75% and 80% reduction in EGFR expression in HCT-116 and Caco-2 cells, respectively, when compared with the corresponding basal levels (Fig. 3).

Although the number of studies performed is relatively small, murine and human studies suggest a role for folic acid in reducing colon carcinogenesis (13, 36). Cravo et al. (10) demonstrated that modest folate deficiency in rats is associated with enhanced development of colon tumors in dimethylhydrazine-treated rats. Alternatively, folate supplementation in this model protected against the development of colonic neoplasms in a dose-dependent manner (20). Moreover, recent human studies noted significantly lower colonic mucosal concentrations of folate in subjects with adenomatous polyps compared with those with hyperplastic polyps despite insignificant differences in serum folate levels (15). Recently, Meenan et al. (33) found folate levels to be lower in adenoma and carcinoma than in normal-appearing adjacent mucosa, suggesting that folate acid may indeed play a key role in the development of colorectal neoplasia.

The mechanisms responsible for the reduction of colon carcinogenesis by folic acid are speculative but may be related in part to the requirement of folic acid in DNA methylation and cellular homeostasis. Folate deficiency may, therefore, result in a variety of cellular consequences, including misincorporation of uracil for thymidine during DNA synthesis (45), resulting in increased spontaneous mutation (44), as well as chromo-
somal abnormalities and errors in DNA synthesis (11, 17). The restoration of DNA methylation status in patients with colorectal neoplasms treated with supra-physiological doses of folic acid (9) lends further support to this hypothesis.

Our current data suggest that supplemental folic acid may have a role in modulating cellular hyperproliferation, an event that is considered central to the initiation of carcinogenesis in the gastrointestinal tract (25). This interpretation comes from the observation that exposure of colon cancer cell lines, Caco-2 and HCT-116, to supplemental folic acid results in inhibition of proliferation in a dose-dependent manner. Furthermore, pretreatment of both cell lines with supplemental folic acid completely abrogated the TGF-α-induced stimulation of cell proliferation. These observations are in agreement with our earlier findings that supplemental folic acid also suppressed carcinogen-induced ODC and tyrosine kinase activities in rats (35). Furthermore, our observation that folic acid is equally effective in inhibiting proliferation of Caco-2 and HCT-116 cells, which are morphologically and phenotypically different, suggests that supplemental folic acid can inhibit proliferation in different colon cancer cells. However, this observation is contrary to what has been noted for normal renal tubular cells, whose proliferation has been shown to be accelerated following a bolus injection of folic acid in rats (31). Although the reasons for this discrepancy is not fully understood, it is plausible that the responsiveness of highly proliferative cancer cells, especially colon cancer cells, to supplemental folic acid is quite different from that of normal cells. The current observation of the folic acid-induced inhibition of proliferation of colon cancer cells could not be attributed to cellular toxicity, since removal of folic acid from the medium after 24 h reverses the inhibition with restoration of proliferative activity (data not shown).

Although the regulatory mechanisms for folic acid-induced inhibition in proliferation of colon cancer cell lines are not fully known, our observation that supplementation of this vitamin also inhibits tyrosine kinase activity suggests a role for these enzymes in regulating the folic acid-induced inhibition of proliferation. Numerous studies suggest that tyrosine kinases play a crucial role in regulating proliferation, differentiation, and transformation of cells (16, 47). However, tyrosine kinases are associated with products of many protooncogenes and with receptors of a number of growth factors (47). Increased activity of several Src-related tyrosine kinases, including pp60c-src, pp56c-fc, and c-Yes has been reported in certain premalignant colon conditions and in colon carcinoma (8, 38, 43). Tyrosine kinase associated with EGFR also appears to be involved in the induction and progression of colorectal neoplasia. We have reported increased EGFR tyrosine kinase activity 10.220.33.2 on October 13, 2017 http://ajpcell.physiology.org/ Downloaded from Fig. 1. Effect of increasing concentrations of folic acid on proliferation of HCT-116 (A) and Caco-2 (B) colon cancer cell lines, which were maintained in RPMI 1640–10% fetal bovine serum (FBS) containing 1 µg/ml folic acid. Aliquots of cell suspension containing 7.5 × 10^4 cells were plated in 24-well culture plates. After 24 h at 37°C, they were incubated for another 48 h in the absence (basal) or presence of increasing concentrations of folic acid. Values represent means ± SE of 5–6 observations.

Fig. 2. Effect of folic acid (FA) pretreatment on transforming growth factor-α (TGF-α)-induced proliferation of serum-starved HCT-116 and Caco-2 cells. Aliquots of Caco-2 (A) and HCT-116 (B) cells, maintained in RPMI 1640–10% FBS and 1 µg/ml folic acid, were incubated for 48 h in the absence (control) or presence of supplemental folic acid (1.25 µg/ml). Aliquots of control and folic acid-treated cells were then serum starved (0.1% FBS) for 24 h and subsequently incubated for another 24 h in the absence or presence of TGF-α (10^-10 M). Values represent means ± SE of 5–6 observations. *P < 0.001, compared with corresponding controls. †P < 0.001, compared with corresponding controls and folic acid-pretreated cells.
activity in the colonic mucosa from patients with ulcerative colitis, adenomatous polyps, and colon cancer (29). In rats, a single injection of the colonic carcinogen azoxymethane markedly stimulates EGFR tyrosine kinase activity and proliferative processes in the colonic mucosa, and both parameters are inhibited by tyrphostin (40). Others have noted that monoclonal antibody to EGFR inhibits proliferation in colon cancer cell lines (18). Considering that activation of EGFR is an important event in the development and progression of colonic carcinogenesis, our observation that folic acid-induced inhibition of proliferation is accompanied by a concomitant reduction in EGFR tyrosine kinase activity suggests that this vitamin may exert its antiproliferative role by modulating EGFR tyrosine kinase.

A number of factors may regulate the intrinsic tyrosine kinase activity of EGFR. The fact that the relative concentrations of EGFR in both Caco-2 and HCT-116 cells, as assessed by Western immunoblot, are greatly decreased following folic acid exposure suggests that decreased activation of the enzyme in response to folic acid may be partly the result of reduced levels of the enzyme protein. Whether the latter is the result of decreased synthesis of EGFR, however, remains to be determined. TGF-α, one of the primary ligands of EGFR, may also modulate the intrinsic tyrosine kinase of EGFR. Because ligand binding is one of the primary causes of activation of EGFR signaling pathways (42), any event(s) that affects the ligand-receptor interaction is likely to have a profound effect on EGFR function. In general, expression of TGF-α is greatly increased in colon cancer cells and may be partly responsible for increased activation of EGFR in colonic neoplasia (1, 3, 18). However, TGF-α is a membrane-bound peptide and the transmembrane precursor form(s) of the peptide is...
able to activate EGF-R through an autocrine/juxtacrine mechanism (22, 30, 36). Our observation that folic acid-induced inhibition of EGF-R tyrosine kinase activity in colon cancer cell lines is associated with a concomitant reduction in the relative concentration of the 14-kDa precursor form of TGF-α in membranes suggests that the peptide might be partly responsible for modulating EGF-R tyrosine kinase through an autocrine/juxtacrine mechanism. Although it remains plausible that other tyrosine kinases may also be involved in regulating the folic acid-induced attenuation of proliferation of colon cancer cells, the fact that supplemental folic acid not only inhibits the expression and activation of EGF-R but also diminishes membrane accumulation of TGF-α in colon cancer cells strongly suggests a key role for EGF-R tyrosine kinase in modulating the antiproliferative effect of folic acid.

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