Expression of glutathione S-transferases (GSTs) in human colon cells and inducibility of GSTM2 by butyrate

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The glutathione S-transferases (GSTs) are a multigene family of enzymes largely involved in the detoxification of chemicals. In animals, enhanced expression is mediated by products of gut fermentation. Of these, butyrate induces GSTP1 protein expression and GST activity in the human colon tumor cell line HT29. In the aim of the following investigations was to further elucidate butyrate-modulated induction of additional colonic GSTs in HT29 and to determine baseline expression in non-transformed cells, isolated from human colorectal tissue. We measured five GST protein subunits (GSTA1/2—composed of GST A1-1, A1-2 and A2-2—GSTM1, GSTM2, GSTP1, GSTT1) by western blot, GST activity using 1-chloro-2,4-dinitrobenzene as substrate and GSTM2 mRNA expression with RT–PCR. GSTP1, followed by GSTT1, were major subunits in all colon cells. Cells isolated from colon tissue were identified to be colonocytes and colon fibroblasts, both of which also expressed substantial levels of GSTM1 and GSTM2. The inter-individual variation of GST subunits in colonocytes of 15 individuals was marked, with total GST protein per 10⁶ cells differing by more than a factor of four. In HT29, butyrate significantly enhanced GSTA1/2 (3.5-fold), GSTM2 (not detectable in controls), GSTP1 (1.5-fold) and GST activity (1.4-fold), but not GSTM1 or GSTT1. GSTM2 mRNA expression was significantly induced after 24 (≈ 14-fold) and 72 h treatment (≈ 8-fold). In colon fibroblasts, butyrate (4 mM, 72 h) also induced GSTM2 protein (1.7-fold) and GST activity (1.4-fold). Colonocytes were too short lived to be used for inducibility studies. In conclusion, GSTs are expressed with high inter-individual variability in human colonocytes. This points to large differences in cellular susceptibility to xenobiotics. However, butyrate, an important luminal component produced from fermentation of dietary fibers, is an efficient inducer of GSTs and especially of GSTM2. This indicates that butyrate may act chemoprotectively by increasing detoxification capabilities in the colon mucosa.

Introduction

Susceptibility to colorectal cancer is associated with several environmental and dietary risk factors, which may be metabolized and detoxified by phase II enzymes, such as glutathione S-transferases (GSTs; EC 2.5.1.18) (1). GSTs are a multigene family of dimeric enzymes that inactivate carcinogens by catalyzing the conjugation of electrophiles to glutathione (2). They are divided into two microsomal and numerous cytosolic GST-classes (alpha, mu, kappa, pi, sigma, theta and zeta) (3). Each class consists of several isoenzymes, with only partly overlapping substrate specificities (4). Extensive deletions in GSTM1 and GSTT1 result in complete loss of enzyme function, which possibly influence colorectal cancer susceptibility (5). Therefore, a high number of studies have been performed to assess whether GSTM1-deficiency or other GST polymorphisms are associated with colorectal cancer susceptibility (6–9). The results were heterogenous and numerous studies failed to demonstrate significant associations. One reason for this could be variations of the absolute expression levels of the GSTs. If these are large they are expected to mask differences due to null polymorphisms. Variations of GST expression in the human colon have not been investigated adequately, but on the basis of data from animal experiments it is known that the expression levels of GSTs are complex, tissue and cell specific (10–12). Moreover, it has been demonstrated in a very great number of animal experiments that they are modulated by numerous and very heterogenic dietary factors (2,13). In the colon, dietary fiber and the resulting fermentation products such as butyric acid could favorably modulate gene expression in cells of the colonic mucosa (14). Several studies have shown that an up-regulation of luminal butyrate concentrations, as a result of microbial carbohydrate fermentation, is associated with a decreased incidence of aberrant crypt foci and colon cancer in rats (15–17). Butyrate inhibits proliferation and induces apoptosis and differentiation in transformed cells, which indicates that it might play an important role in the secondary prevention of colon cancer (18,19). Additionally, butyrate could contribute to risk reduction by enhancing expression of GSTs that protect colon cells from endogenously formed oxidation products 4-hydroxy-2-nonenal (20). In this context, we have shown that butyrate increases GSTP1 expression in transformed human colon cells, which is the major isoform in the colon (21). However, in view of multidrug resistance, which may be caused in part by increased GSTP1 levels in tumor cells, the interpretation of this finding is under debate in terms of potential positive effects (22). Therefore, the aim of this paper was to examine expression profiles of additional GST isoenzymes, e.g. of the alpha, mu and theta classes, how they are affected by butyrate and whether similar effects as reported before for GSTP1 in tumor cells also occur in non-transformed cells. In particular, we determined protein expression of GSTA1/2, GSTM1, GSTM2, GSTP1 and GSTT1 as well as the overall activity of GST. First, we analyzed the...
baseline expression levels of the GSTs in primary colon cells derived from 15 individuals to assess variability of expression levels and compared these with baseline levels in primary colon fibroblasts and in HT29 colon tumor cells. Next we incubated the cells with butyrate and determined the various GST proteins as well as GSTM2 mRNA.

Materials and methods

Cell lines
The human colon carcinoma cell line HT29 was grown in culture medium [DMEM, Gibco BRL, Karlsruhe, Germany; supplemented with 10% (v/v) fetal calf serum and 1% (v/v) penicillin/streptomycin] at 37°C in a humidified incubator (5% CO2/95% air), as specified previously (23). Human colon fibroblasts were isolated from two different patients (see below) termed as cultures 1 and 2 and subsequently cultivated using the same conditions as described for HT29. Passages 17–46 of HT29 and 6–10 of the fibroblast cell lines were used for the experiments.

Cell treatment for analyses
HT29 and fibroblasts were incubated with 4 nM Na–butyrate (99% purity, Merck, Darmstadt, Germany) dissolved in culture medium in culture flasks. This concentration is based on concentrations found to be effective previously (20). After 48 h of incubation, the cells were trypsinized and washed in cold phosphate-buffered saline (20). Cell numbers were counted and viability was determined using trypan blue exclusion. The cells were frozen in liquid nitrogen and stored at −80°C prior to preparation of cytosolic fractions and isolation of RNA and DNA.

Preparation of tissue
The donors of colon tissue were admitted to the university hospital (Jena, Germany) for surgical removal of colorectal tumors, diverticulitis or colon polyps. Parts of the excised non-tumorous tissue, removed together with the tumor or other pathology samples, were used for cell isolation. The Ethical Committee of the Friedrich-Schiller-University of Jena approved the study and the patients have given their informed consent. Tissue specimens of 17 donors were studied (mean age, 60 years; range, 30–81 years). The donors were 10 males and five females. For two samples, information on gender was missing. For basal expression of GST isoenzymes, tissue specimens of 15 donors were examined. Six of these specimens were additionally incubated with butyrate for 24 h in DMEM. Two additional specimens were used only for isolation of fibroblasts.

The newly established procedure to isolate colon cells from surgical samples has been described in detail recently (24). Briefly, tissue was stored in Hanks balanced salt solution (HBSS) and transported on ice to the laboratory within 1 h. Sections of epithelial tissues (±0.3–0.5 cm²) were separated from the underlying tissue by perfusion-supported mechanical disaggregation. The epithelial stripes were either worked up immediately (for determination of basal GST expression) or were incubated with 4, 10 and 20 mM butyrate in culture medium ± 2% (v/v) penicillin/streptomycin for 24 h at 37°C (for determination of GST induction). The cell slurries were worked up by mincing and incubation (40–60 min, 37°C) in HBSS supplemented with 1 mg/ml collagenase P (Boehringer Mannheim, Mannheim, Germany) (25). The suspensions were diluted with HBSS, centrifuged and washed with cold HBSS. Cell numbers were counted and viability was determined using trypan blue. Cells were frozen in liquid nitrogen and stored at −80°C prior to further use.

For isolation of fibroblasts, sections of epithelial tissue were cut into small pieces of 1 mm², which were placed in a culture flask and were allowed to grow on the bottom of the flask for 1 h in the incubator. Afterwards, culture medium [with 2% (v/v) penicillin/streptomycin] was added and fibroblasts were allowed to grow for at least 3 weeks. Then the fibroblasts were typsinized and washed in cold phosphate-buffered saline (20). Cell numbers were counted and viability was determined using trypan blue. The cells were frozen in liquid nitrogen and stored at −80°C prior to further use.

GST activity and cytosolic protein

GST activity with 1-chloro-2,4-dinitrobenzene (CDNB) as substrate was determined spectrophotometrically at 340 nm and 30°C (26). Total protein content was measured using the method by Bradford with bovine serum albumin as standard protein (27).

Western blot analysis of GST isoenzymes

Cytosols and GST protein standards were diluted (1:1.5) with loading buffer [125 mM Tris, 2% sodium dodecylsulfate (SDS), 10% glycerine, 6 M urea, 324 mM dithiothreitol, 0.1% bromophenolblue] and incubated for 10 min at 65°C. Samples and standards were subjected to SDS–polyacrylamide gel electrophoresis (stacking gel, 3% w/v acrylamide; separation gel, 12% w/v acrylamide) and transferred to nitrocellulose (Schleicher & Schuell, Dassel, Germany) in a semi-drying blotting chamber (MTV1, Cili, GmbH, Idstein/Taunus, Germany). For detection of GST isoenzymes, defined amounts of cytosolic protein were loaded (HT29: 60 μg protein for GSTA1/2, M1, M2 and 8 μg protein for GSTP1, GSTT1; fibroblasts: 10–20 μg protein and primary colon cells: 5–15 μg protein for all GSTs). The western blots were subsequently blocked with milk protein (5%) and incubated with the appropriate antibody. We used monoclonal antibodies against human GST class alpha, mu, pi (21) and theta class (LabAs, Tartu, Estonia) (30). While class pi and class theta antibodies are directed against GSTP1 and GSTT1, the class alpha antibodies recognize GSTA1-1, GSTA1-2 and GSTA2-1 (both Calbiochem, Darmstadt, Germany) and were run in parallel and served as standards for calculating total amounts of the isoenzymes in the samples.

GST polymorphisms in primary human colon cells

Null polymorphisms of genes for GSTM1 and GSTT1 were determined as described previously (30), by multiplex polymerase chain reaction (PCR) using original procedures (31,32).

RNA preparation and semi-quantitative reverse transcriptase–PCR (RT–PCR)

Total RNA was isolated from HT29 using the TRIzol method (Gibco BRL). Following DNA digestion (Deoxyribonuclease I, Amplification Grade, Invitrogen, Karlsruhe, Germany) cDNA equivalent to 5 μg total RNA was prepared by first-strand synthesis using oligo(dT) primer (SuperScriptII First-Strand Synthesis System, Gibco BRL). Semi-quantitative RT–PCR was used to amplify GSTM2 mRNA levels and to compare its expression in butyrate treated cells versus untreated cells. The expression of glycinealdehyde-3-phosphate-dehydrogenase (GAPDH) served as an internal control. To avoid different cDNA concentrations between target gene (GSTM2 and control gene (GAPDH), for each sample the template (CDNA) was added to the PCR reaction mix without primers. Subsequently the mixture was aliquoted in two batches, one for GSTM2 and one for GAPDH, before adding primers, which had the following sequences for GSTM2 (sense)

5'-TCC ACC ACC CGT TTG CTG TAG-3' and for GSTM2 (antisense), respectively: 5'-GGG AAT CGA AAA AGG AGC AG-3' and 5'-CAC GAG AAA GGA ACG AG-3', and for GAPDH (sense) and GAPDH (antisense), respectively: 5'-ACC ACA GTC CAT GCC ATC AC-3' and 5'-TCC ACC ACC CGT TTG CTG TAG-3'. On the basis of the mRNA sequence (NCBI Nucleotide Sequence Database accession number NM_000848 for GSTM2 and J04038 for GAPDH) the predicted size of the GSTM2 (sense) and GSTM2 (antisense), respectively: 5'-GGG AAT CGA AAA AGG AGC AG-3' and 5'-CAC GAG AAA GGA ACG AG-3', and for GAPDH (sense) and GAPDH (antisense), respectively: 5'-ACC ACA GTC CAT GCC ATC AC-3' and 5'-TCC ACC ACC CGT TTG CTG TAG-3'. On the basis of the mRNA sequence (NCBI Nucleotide Sequence Database accession number NM_000848 for GSTM2 and J04038 for GAPDH) the predicted size of the GSTM2 (sense) and GAPDH (antisense), respectively: 5'-GGG AAT CGA AAA AGG AGC AG-3' and 5'-CAC GAG AAA GGA ACG AG-3', and for GAPDH (sense) and GAPDH (antisense), respectively: 5'-ACC ACA GTC CAT GCC ATC AC-3' and 5'-TCC ACC ACC CGT TTG CTG TAG-3'. On the basis of the mRNA sequence (NCBI Nucleotide Sequence Database accession number NM_000848 for GSTM2 and J04038 for GAPDH) the predicted size of the GSTM2 and GAPDH. The PCR cycles were run at 95°C for 15 s, 55°C for 30 s and 72°C for 1 min. For quantitative RT–PCR the number of PCR cycles was calibrated for GAPDH as internal control (15–41 cycles) and 41 cycles were used for GSTM2. Densitometry evaluation of the ethidium bromide bands was performed with the Quantity One 4.1 Software (Bio-Rad). The results were expressed as ratio of GSTM2 and GAPDH.

Statistical evaluation

Data were based on both the cytosolic protein content (per mg protein) and on the number of cells (per 10⁶ cells), respectively. All values given are...
Results

Basal expression of GST isoenzymes in HT29, primary colon cells and colon fibroblasts

Cellular levels of GST protein expression were calculated both on the basis of number of cells and on the cytosolic protein (Table I). Determinations were performed after cultivating HT29 and colon fibroblasts for 5 and 10 days. Values of primary colon cells were obtained from tissue specimens worked up within 3 h after surgery.

GSTP1 was the major GST subunit in all colon cells, including the fibroblasts, whereas GSTA1/2 was expressed least. GSTA1/2 was detectable in nine of 11 samples of primary colon cells, but was not found in either of the two colon fibroblast cultures. In HT29, GSTM1 and GSTM2 proteins were not detectable, although HT29 cells do not bear the null-genotype for GSTM1 (20,33). In contrast, both primary colon cells and colon fibroblasts express considerable amounts of GSTM1 and GSTM2. GSTT1 protein was detected in all cells, unless the GSTT1*0 polymorphism was identified. This was the case for one of the fibroblast cultures, whereas the other was GSTM1*0 (Table I). Figure 1 shows the variation for GST subunit expression in primary colon cells obtained from 15 different donors. Null polymorphisms were identified in several samples not expressing GSTM1 (n = 4 of 7) or GSTT1 (n = 2). Altogether, the total protein contents varied from 250 to 930 ng GST protein/10⁶ cells. Mean tertoil values were 719 ± 142, 417 ± 34 and 315 ± 42 ng/10⁶ cells (or 6088 ± 1154, 3244 ± 629 and 2109 ± 331 ng/mg protein), which is equivalent to more than a 2–3-fold difference of GST expression in human colonocytes.

Butyrate-mediated induction of GST subunits in HT29

To establish optimal in vitro conditions of GST inducibility, we first determined protein expression after incubating HT29 cells with butyrate for 24, 48 and 72 h. Treatment with butyrate enhanced protein levels of GSTA1/2, GSTM2 and GSTP1 in a time-dependent manner, whereas GSTM1 and GSTT1 levels were not influenced. The increase of GSTM2 was already detectable after 24 h and of GSTA1/2 after 48 h incubation time (results not shown). Table II shows the maximal levels of induction that were apparent after 72 h with significant increases for GSTA1/2 (3.5-fold), GSTM2, GSTP1 (1.5-fold) accompanied by 1.4-fold increase of GST activity. The calculation of induction values for GSTM2 (all time points) and GSTA1/2 (after 48 h) was not possible, as these isoenzymes were not detectable in their respective controls. Representative examples of immunoblots, detecting GSTA1/2, GSTM2, GSTP1 and GSTT1 are shown in Figure 2. This figure also demonstrates that GST inductions are already apparent for only 2 mM butyrate.

GSTA1/2 and GSTP1 levels of controls also increased with longer cultivation time. The increase of GSTP1 from 2395 ± 595 (24 h) to 5855 ± 535 (72 h) ng/mg total protein was significant. GSTA1/2 protein became detectable in controls after 72 h.
Table 1. Quantification of basal levels of GST subunits, GST activity and total protein content in human colon cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>GST protein (ng)</th>
<th>GST activity (nmol (\text{min}^{-1}))</th>
<th>Total protein (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10(^{-6}) cells mg(^{-1}) protein</td>
<td>10(^{-6}) cells mg(^{-1}) protein</td>
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<tr>
<td></td>
<td></td>
<td>10(^{-6}) cells mg(^{-1}) protein</td>
<td>10(^{-6}) cells mg(^{-1}) protein</td>
</tr>
<tr>
<td>A1/2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HT29 ((n = 17))</td>
<td>1.2±0.3</td>
<td>805±252</td>
<td>40±7</td>
</tr>
<tr>
<td>Colon fibroblasts ((n = 3))</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Colon fibroblasts ((n = 5))</td>
<td>n.d.</td>
<td>GSTM1(^{-})0</td>
<td>2797±743</td>
</tr>
<tr>
<td>Primary colon cells ((n = 11\–15))</td>
<td>17±15</td>
<td>964±283</td>
<td>3050±406</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD. n.d., not detectable. GSTM1\(^{-}\)0 and GSTT1\(^{+}\)0, null-polyorphism genotyping.
Our data show a time-dependent up-regulation of GSTA1/2, GSTM2 and GSTP1 protein by butyrate, but no change of GSTT1 in the human colon tumor cell line HT29. It would be a matter of perception to describe the increase of GSTP1 as a marker of chemoprevention, as it is also discussed as being a tumor marker due to its over-expression in colon carcinoma (42). However, the increases of GSTA1/2 and GSTM2, which are down-regulated in tumor cells (21), could very well be interpreted as contributing to the differentiation of the transformed cells. The inducibility of GSTP1 was relatively small compared with the degree of induction for GSTA1/2 and GSTM2, which can be explained by the already high basal expression of this isoenzyme. Despite the remarkable induction of GSTA1/2 and GSTM2 protein, the absolute amounts of these isoenzymes still represented < 1% of detected GSTP1 protein. Therefore, the induction of overall GST activity was probably mainly due to the increase of GSTP1 levels. The increase of GST protein with cultivation time that we observed for GSTA1/2 and GSTP1 corresponds to earlier results with the human colon carcinoma cell line Caco-2 (43).

The butyrate-mediated enhancement of GSTM2 mRNA confirms the western blot data on induced GSTM2 protein levels. Possible explanations are that butyrate could either enhance the transcriptional activity of the gene or improve the stability of GSTM2 mRNA. In any case, this is the first time that a butyrate-mediated increase of GSTM2 has been demonstrated in human colon cells. An induction of this isoenzyme by the green tea polyphenol epigallocatechin gallate was recently found in rat liver, where it also enhanced overall GST activity (44). GSTM2 has a high specific activity toward CDNB, the standard substrate of activity measurement (3,26). In vivo GSTM2 is known to efficiently detoxicate o-quinones (e.g. aminochrome), the oxidation products of catecholamines, which may be involved in the development of the Parkinson's disease (45). Furthermore, noticeable activities of hepatic rat GSTM2 towards 4-hydroxy-2-nonenal were found (46), which may be of importance for carcinogenesis (47,48). However, it is not clear which putative colon risk factors are specifically detoxified by GSTM2. Moreover, it is also not known how the induction of GSTM2 mRNA levels occur on a molecular level.

The murine GSTM2 promoter region does not contain a TATA-box and activation of the gene is based on an SP-1 binding sites (49). The binding sites for transcription factors of the human GSTM2 gene have not been characterized yet. In primary colon cells GSTs are expressed differently than in the tumor cell line HT29. Thus, primary colon cells expressed GSTM1 and GSTM2 protein in considerable amounts, provided that no null-deletion for GSTM1 was present. Also, the GSTA1/A2 levels on the average were several times higher than in HT29 cells (21). The relatively high levels of these GSTA are interesting in terms of colon cancer risk, as several putative colon carcinogens can be specifically deactivated by these enzymes, such as N-acetoxy-2-amino-1-methyl-6-phenylimidazo[326x423]...
[4,5-b]pyridine (N-acetoxy-PhIP) by GSTA1-1 (50). Total GSTs are also expressed with a marked intraindividual variability—some of the samples containing 2–4-fold higher GST protein levels than others. In at least three of the investigated tissues (from donors 7, 12 and 13), not even GSTP1 was available in abundant quantities (<100 ng/10^6 cells). Another interesting find was that cells with the GSTM1-null genotypes do not necessarily compensate their deficiency by higher expression levels of the other GST enzymes, a finding which confirms previous reports (51–53). On the basis of our present knowledge, these findings mean that a considerable number of subjects could be at higher risk on account of low GST expression levels. Whereas many studies have been published on associations of polymorphisms and colon cancer risk, hardly any are available on the associations of expression levels and cancer risks, probably due to technical limitations. Technical limitations were also the reason why we could not study an additional butyrate-mediated GST-protein-induction in the primary colon epithelial cells, as they do not survive long enough. Adult colon epithelial cells can be considered as models for the actual target cells of cancer, which are the stem cells and their migrating daughter cells localized in the lower crypt segments (54). It is not known to which percentage stem cells and their immediate daughter cells are contained in our mixed cellular suspensions as they cannot easily be identified, but their proportion is probably variable and rather low (55). On the other hand, the expression levels in the adult colonocytes are probably also very important for protecting the stem cells from genotoxic insults. Luminal genotoxins could be warded off, by first being detoxified in the more mature cells of the upper crypt of the mucosal epithelium. Moreover, the colon epithelium consists not only of enterocytes and seam cells—both termed as colonocytes—but is also surrounded by connective tissue containing fibroblasts. Fibroblasts can be involved in the human body’s defence by adaptive gene expression (56) and by detoxifying xenobiotics through enhanced GST expression (57). Thus, the enzyme pattern of fibroblasts in the colon could be adapted to the surrounding colon tissue and together with the colon tissue protect the stem cells from genotoxic insults. Butyrate treatment of two non-transformed fibroblast cultures (4 mM; 72 h) increased the already strongly expressed GSTM1 and GSTM2 protein levels.

On the basis of these results, we suggest that butyrate modulates GST expression in colon cells, especially GSTM2, and that this enhanced expression in adult colonocytes and fibroblasts can protect not only the cells themselves, but also the actual target cells of cancer, which are located in the bottom portions of the colon crypts. An enhancement of GSTP1 by resistant starch (14) or GSTA1/2 by wheat bran (58) has been reported and attributed to increased luminal butyrate concentrations. In spite of the extensive literature on the various mechanisms by which butyrate could act as a chemopreventive compound, its in vivo potentials of protective activities are hardly elucidated. Butyrate-mediated induction of cellular protein content, as we found, indicates an up-regulation of a large number of genes (59), possibly due to the modification of histone acetylation (60). Also, we have shown that butyrate activates ERK1/2 (20), but further studies

Fig. 2. Sample blots showing the induction of GSTA1/2, GSTM2, GSTP1 and GSTT1 in HT29 cells after 72 h treatment with 2–4 mM butyrate.

Fig. 3. Induction of GSTM2 mRNA expression based on GAPDH mRNA in HT29 after 24 and 72 h butyrate treatment, respectively. Trace quantity as ratio of GSTM2/GAPDH is shown (mean ± SD; control versus treatment; *P < 0.05, **P < 0.01, significant in two-tailed unpaired t-test, n = 4).
are needed to clarify how GSTM2 or GSTP1-gene activation is caused at the molecular level.

In conclusion, our study shows that primary human colon cells express different GST proteins with large inter-individual variability. The expression patterns are also highly different from transformed HT29 colon tumor cells, particularly in that GSTM2 is more abundant. The luminal factor butyrate specifically induces GSTM2 in two types of colon cell cultures and could thus be the source of enhanced GSTM2 levels in adult colonocytes. It is an isoenzyme with specificity for substrates that arise from oxidative stress. Therefore, it may well be possible, that dietary-mediated butyrate formation in the gut will modulate GSTs of the colon mucosa in a favorable manner, leading to a reduced impact of certain genotoxic colon cancer risk factors and thus enhancing lifelong protection in the general population.

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