Iron deficiency and iron chelators are known to alter folate metabolism in mammals, but the underlying biochemical mechanisms have not been established. Although many studies have demonstrated that the iron chelators mimosine and deferoxamine inhibit DNA replication in mammalian cells, their mechanism of action remains controversial. The effects of mimosine on folate metabolism were investigated in human MCF-7 cells and SH-SY5Y neuroblastoma. Our findings indicate that mimosine is a folate antagonist and that its effects are cell-specific. MCF-7 cells cultured in the presence of 350 μM mimosine were growth-arrested, whereas mimosine had no effect on SH-SY5Y cell proliferation. Mimosine altered the distribution of folate cofactor forms in MCF-7 cells, indicating that mimosine targets folate metabolism. However, mimosine does not influence folate metabolism in SH-SY5Y neuroblastoma. The effect of mimosine on folate metabolism is associated with decreased cytoplasmic serine hydroxymethyltransferase (cSHMT) expression in MCF-7 cells but not in SH-SY5Y cells. MCF-7 cells exposed to mimosine for 24 h have a 95% reduction in cSHMT protein, and cSHMT promoter activity is reduced over 95%. Transcription of the cSHMT gene is also inhibited by deferoxamine in MCF-7 cells, indicating that mimosine inhibits cSHMT transcription by chelating iron. Analyses of mimosine-resistant MCF-7 cell lines demonstrate that although the effect of mimosine on cell cycle is independent of its effects on cSHMT expression, it inhibits both processes through a common regulatory mechanism.

There are several well characterized cellular responses that are triggered following decreases in the regulatory, non-ferritin-bound iron pool. Many of these responses are mediated through the iron regulatory protein, which can bind with specificity to certain mRNA species and regulate translation (1). The concentration of cellular regulatory iron is decreased by iron deficiency or by elevated expression of heavy chain ferriportin, a protein that sequesters intracellular iron (2, 3). Sequestration of intracellular iron by chelators, including DFO, has been commonly used to trigger physiological changes associated with iron deficiency. The influence of iron deficiencies, both induced and naturally occurring, on folate metabolism has been well documented in cell culture models, animal models, and humans. Iron deficiency can result in morphological alterations in granulocytes similar to folate deficiency (4), and iron deficiency has been demonstrated to impair folate utilization in some but not all tissues (5). In addition, maternal iron deficiency decreases secretion of folate into milk (6, 7) without decreasing maternal serum or red blood cell folate levels in rats. However, the biochemical mechanisms underlying the influence of iron deficiency on folate metabolism have not been established (6).

Mimosine, a plant amino acid and tyrosine analog (Fig. 1), is a toxin that chelates iron and inhibits mammalian DNA replication. Mimosine is known to block DNA replication in breast cancer cells and Chinese hamster ovary cells at the initiation phase, although, the precise mechanism by which mimosine alters DNA replication remains unclear (8). Recently, Alpan and Pardee (11) proposed a mechanism to account for the effects of mimosine on DNA replication. This mechanism proposes that mimosine induces a cascade of events that result in the inhibition of DNA replication. This cascade is initiated by mimosine targeting ribonucleotide reductase (RNR) or the folate-dependent enzyme SHMT, resulting in an inhibition of dNTP synthesis. There is some evidence to support this suggestion. Mimosine decreases purine deoxyribonucleotide pools over 85% in Chinese hamster ovary cells but does not affect pyrimidine deoxyribonucleotide pools (9). The decrease in dNTP pools is also observed upon treatment with the iron chelator DFO. This effect has been attributed to the inhibition of the iron-dependent enzyme RNR (10) and subsequent disruption of purine deoxyribonucleotide biosynthesis. However, other data do not support this mechanism. Inhibition of RNR would not be expected either to inhibit the initiation phase of DNA replication (8) or to specifically lower purine deoxyribonucleotide pools. Furthermore, the effect of mimosine on DNA replication is cell-specific. Embryonic Xenopus and mouse cells are resistant to mimosine (9, 11, 12). Inhibition of RNR by iron chelation should not display cell specificity, and therefore, mimosine resistance would not be expected.

Mimosine has also been suggested to deplete purine deoxyribonucleotide pools by targeting folate metabolism through the inhibition of the enzyme serine hydroxymethyltransferase (SHMT). SHMT catalyzes the conversion of tetrahydrofolate (THF) and serine to glycine and methyleneTHF (14). This reaction generates single carbon units that are carried by folate cofactors for purine, thymidine, and methionine biosynthesis (14) (Fig. 2). Mimosine has been shown to bind SHMT in crude Chinese hamster ovary cell extracts (13). However, mimosine does not inhibit SHMT activity in vitro. Therefore, the role of mimosine in influencing SHMT function and the role of SHMT in DNA replication inhibition has yet to be proved (13).

The second step in the mechanism put forth by Alpan and Pardee (11) proposes that depletion in dNTP substrates results in DNA strand breaks, which induce the expression of p21, a
The precipitating event in this cascade is the formation of DNA strand breaks caused by inhibition of SHMT and folate metabolism or RNR by mimosine. However, there is little conclusive evidence that mimosine influences folate metabolism or RNR activity in vivo. In the current study, we examine the effects of mimosine and another iron chelator, DFO, on SHMT and folate metabolism. These studies indicate that mimosine does target folate metabolism, but in a cell-specific manner. We propose that the effects of mimosine on folate metabolism do not directly result in an inhibition of DNA replication. Rather, we demonstrate that mimosine can alter cSHMT gene expression and thereby modify folate metabolism in a cell-specific manner, and we provide evidence that there is a regulatory transcriptional mechanism that is induced by mimosine that accounts for the cell-specific effects of mimosine on cell cycle progression.

Fig. 1. Structure of mimosine and tyrosine. Mimosine is a tyrosine analog and an iron chelator.

Fig. 2. Overview of cytoplasmic folate metabolism. The three primary products of cytoplasmic folate metabolism are purines, thymidylate, and methionine. The formate is derived from mitochondrial folate metabolism (25). The primary enzymes discussed include cSHMT, MTHFR, and MS.
Schematic of luciferase activity generated from the co-reporter, pRL-CMV from either construct (pcSHMT-luc or pcSHMT-SV40-luc) was normalized to luciferase activity generated from the cell monolayers containing 5 × 10^6 cells were washed three times with phosphate-buffered saline and detached from the culture plate by scraping. Cells were lysed with 1 ml of 50 mM potassium phosphate, pH 7.2, containing 0.1% Triton X-100. SHMT activity was determined in the crude cell extracts by measuring the rate of exchange of the pro-2S proton of [2-3H]glycine, as described previously (20). This assay measures both mitochondrial and cytoplasmic SHMT activity. All assays were performed in triplicate, and variance is expressed as the standard error of the mean.

Western Blot Analyses—MCF-7 cells were cultured in the presence of the DNA replication inhibitors mimosine (350 μM), aphidicolin (6 μM), or hydroxyurea (1 mM). Cells were lysed with 1 ml of 50 mM potassium phosphate, pH 7.2, containing 0.1% Triton X-100. SHMT activity was determined in the crude cell extracts by measuring the rate of exchange of the pro-2S proton of [2-3H]glycine, as described previously (20). This assay measures both mitochondrial and cytoplasmic SHMT activity. All assays were performed in triplicate, and variance is expressed as the standard error of the mean.

**Folate Metabolism and Iron**

**Mimosine Targets Tumor Cell Lines with Specificity—**Several studies have suggested that mimosine inhibits cell cycle progression by depleting purine deoxyribonucleotide pools through the inhibition of the folate-dependent enzyme SHMT (13). Previously, we have characterized folate metabolism in both cultured human MCF-7 cells and SH-SY5Y neuroblastoma (16, 22) and have used these cell lines to study the role of SHMT in folate metabolism. Whereas mimosine has been demonstrated to inhibit both human breast cancer (MDA-MB-453) (10) and Chinese hamster ovary cell proliferation (23), the effect of mimosine on MCF-7 and SH-SY5Y neuroblastoma cell proliferation has never been investigated. MCF-7 cells exposed to 350 μM mimosine incorporated less than 5% of [methyl-3H]thymidine into DNA relative to untreated MCF-7 cells after a 24 h exposure to mimosine (Fig. 3A). The onset of inhibition occurs within the first 24 h of mimosine exposure, indicating that MCF-7 cells are a mimosine-sensitive cell line.

The incorporation of [methyl-3H]thymidine into SH-SY5Y neuroblastoma was not influenced by 350 μM mimosine over 4 days of exposure, demonstrating that mimosine does not inhibit cell cycle in SH-SY5Y neuroblastoma (Fig. 3B). These results are consistent with previous studies that have demonstrated mimosine resistance in embryonic cells and confirm that mimosine is a cell-specific DNA replication inhibitor.

**TABLE I**

| Pretreatment 1, duration | Treatment 2, duration | 10-FormylTHF | THF | 5-FormylTHF | 5-MethylTHF |
|-------------------------|----------------------|--------------|-----|------------|------------|
| None                    | 20 nm [3H]folic acid, 12 h | 50 ± 4       | 12 ± 2 | 6 ± 2 | 25 ± 3 |
| 300 μM mimosine, 1 h    | 20 nm [3H]folic acid, 300 μM mimosine, 12 h | 27 ± 8       | 45 ± 7 | 5 ± 1 | 12 ± 2 |
| 300 μM mimosine, 72 h   | 20 nm [3H]folic acid, 300 μM mimosine, 12 h | 34 ± 2       | 17 ± 3 | 4 ± 2 | 45 ± 3 |

**Fig. 3.** The effect of mimosine of MCF-7 and SH-SY5Y cell proliferation. The rate of [methyl-3H]thymidine incorporation into DNA was determined in MCF-7 cells (A) and SH-SY5Y cells (B). Cells were cultured with αMEM in the absence (triangles) and presence (squares) of 350 μM mimosine as described under “Experimental Procedures.” 24 h prior to harvest, the medium was removed and replaced with medium containing [methyl-3H]thymidine (2.5 μCi/ml). Incorporation of [methyl-3H]thymidine into DNA was quantified using a liquid scintillation counter. All measures were taken in triplicate, and error bars represent S.E.
These results also indicate that mimosine does not inhibit DNA replication by inhibiting RNR, because this mechanism of inhibition would not be expected to display cell specificity.

Mimosine Affects Folate Metabolism with Cell Specificity—The effect of mimosine on the relative distribution of folate one-carbon forms was investigated (Table I) in MCF-7 and SH-SY5Y cells. The folate one-carbon pool is sensitive to disruptions or alterations in individual folate metabolic pathways. Exposure of MCF-7 cells to mimosine for 72 h results in the accumulation of intracellular folate as 5-methylTHF (Table I). 5-MethylTHF accounts for 45% of total intracellular folate in mimosine-treated cells, compared with 25% in untreated MCF-7 cells. This suggests that nearly all cytoplasmic folate is present as 5-methylTHF, because approximately 50% of cellular folate is located in the mitochondria, and mitochondria do not accumulate 5-methylTHF (24). Therefore, mimosine targets enzymatic reactions associated with the regulation of 5-methylTHF concentrations.

The effect of mimosine on folate metabolism is dynamic (Table I). Exposure of MCF-7 cells to 300 μM mimosine for 12 h results in decreased levels intracellular 5-methylTHF. Under these conditions, 5-methylTHF accounts for only 12% of total intracellular folate in mimosine-treated cells. These results show that the effects of mimosine on folate metabolism vary markedly as a function of time between 12 and 72 h. This suggests that the interaction of mimosine with folate metabolism is complex and that homeostatic mechanisms may exist to alter folate metabolism to compensate for the effects of mimosine on folate metabolism.

Treatment of SH-SY5Y cells with mimosine did not influence the distribution of folate cofactors at either the 12 or 72 h measurement (data not shown). Mimosine does not influence folate one-carbon pools in SH-SY5Y cells as was observed in MCF-7 cells, demonstrating that mimosine influences folate metabolism in a cell-specific manner.

Mimosine Targets Serine Hydroxymethyltransferase—The accumulation of intracellular folate as 5-methylTHF in MCF-7 cells resulting from mimosine exposure indicates that mimosine alters 5-methylTHF metabolism. The enzymes that regulate 5-methylTHF include methionine synthase (MS), methylentetrahydrofolate reductase (MTHFR), and cSHMT (Fig. 2). Increased 5-methylTHF levels could result from increased MTHFR activity, decreased MS activity, or decreased cSHMT activity. Because the MTHFR reaction is essentially irreversible (14, 25), inhibition of MS would be expected to result in the accumulation of homocysteine and SAH, and decreased levels of SAM. The methylation of homocysteine to methionine by MS is critical to maintain SAM levels in the cell (14, 26, 27). Therefore, in order to determine whether mimosine targets MS, both SAM and SAH levels were measured in MCF-7 cells prior to and following exposure to mimosine (Table II). Exposure of MCF-7 cells to mimosine does not significantly influence the intracellular SAM or SAH levels, indicating that mimosine does not inhibit MS (Table II). Additionally, mimosine does not inhibit the activity of the E. coli vitamin B12-dependent methionine synthase in vitro. These results show that the effect of mimosine on folate metabolism is independent of MS activity.

Previously, we demonstrated that serine synthesis catalyzed by cSHMT and 5-methylTHF synthesis catalyzed by MTHFR compete for 5,10-methyleneTHF in SH-SY5Y cells (16) (Fig. 2). Therefore, the observed accumulation of 5-methylTHF in MCF-7 cells following mimosine exposure may be due to an inhibition of cSHMT activity and increased 5-methylTHF synthesis. Extracts from MCF-7 cells that were exposed to 350 μM mimosine, for 72 h, displayed a 20% decrease in total SHMT activity compared with untreated MCF-7 cells (Table III), demonstrating that mimosine does decrease the specific activity of SHMT in MCF-7 cells. It is not possible to specifically measure cSHMT activity in these cells, due to the low intracellular concentrations of cSHMT relative to the mitochondrial SHMT isozyme concentrations. We estimate that the cSHMT protein accounts for approximately 25% of the total SHMT activity in MCF-7 cells. The addition of 500 μM mimosine to extracts of MCF-7 cells cultured in the absence of mimosine does not result in decreased SHMT activity, confirming previous studies that mimosine does not inhibit SHMT activity in vitro (13). Therefore, although mimosine does decrease total SHMT-specific activity in MCF-7 cells exposed to mimosine, the decrease in SHMT activity is not directly due to the inhibition of the cSHMT enzyme activity. However, mimosine may alter cSHMT enzyme protein levels.

MCF-7 cells exposed to mimosine for 72 h did not contain detectable amounts of cSHMT protein as determined by Western blots (Fig. 4). Similar results were seen after only a 24-h expo-

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**TABLE II**

| Cell type | Culture medium | SAM ng/mg protein | SAH ng/mg protein |
|-----------|----------------|-------------------|-------------------|
| MCF-7     | αMEM           | 24.7 ± 0.5        | 2.1 ± 1.3         |
| MCF-7     | αMEM + 150 μM mimosine | 19.3 ± 0.3 | 3.8 ± 2.2 |

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**TABLE III**

| Culture conditions | Mimosine in assay | Total SHMT activity (3H exchanged) |
|--------------------|-------------------|-----------------------------------|
| MCF-7              |                   |                                   |
| αMEM               | 0.0               | 16.9 ± 1.0 (100%)                 |
| αMEM               | 500               | 16.0 ± 0.9 (95%)                  |
| αMEM + 350 μM mimosine | 0.0             | 13.6 ± 0.5 (80%)                  |

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**Fig. 4. Effect of mimosine on cSHMT enzyme concentrations in MCF-7 cells.** MCF-7 cells were cultured in αMEM in the presence and absence of DNA replication inhibitors for 70 h. Cells were harvested, and cSHMT protein levels were determined by Western blots as described under "Experimental Procedures." Lane A, MCF-7 cells cultured with αMEM; lane B and C, MCF-7 cells cultured with αMEM containing 350 μM mimosine; lane D, MCF-7 cells cultured with αMEM containing 6 μM aphidicolin; lane E, MCF-7 cultured with αMEM cells containing 1 mM hydroxyurea.
Mimosine Inhibits cSHMT Gene Transcription—Examination of cSHMT mRNA levels by reverse transcription-polymerase chain reaction in MCF-7 cells exposed to 350 μM mimosine for 48 h demonstrated a loss of cSHMT mRNA. Therefore, the effect of mimosine on cSHMT promoter activity was investigated using a luciferase gene reporter assay as described under “Experimental Procedures.” The cloning and initial characterization of the human cSHMT proximal promoter has been reported previously (21). MCF-7 cells cultured with 350 μM mimosine for 24 h prior to transfection with pCSHMT-luc displayed a 95% decrease in normalized luciferase activity relative to MCF-7 cells cultured without mimosine. Mimosine is an effective iron chelator and tyrosine analog, and it has been suggested that mimosine inhibits DNA replication by chelating iron (12). Therefore, the effects of tyrosine and DFO on luciferase activity were determined in MCF-7 cells (Table IV). MCF-7 cells cultured with 150 μM DFO for 24 h prior to transfection with pCSHMT-luc displayed similar decreases in luciferase activity as seen with mimosine pretreatment. Tyrosine pretreatment (350 μM) had no effect on cSHMT promoter activity (Table IV). This suggests that the mimosine-induced decrease in luciferase activity is the result of iron chelation. Mimosine also decreased the relative luciferase activity of pCSHMT-SV40-luc by 75%. This suggests that the cSHMT promoter has a consensus sequence for a silencer that can inhibit SV40 driven transcription of the luciferase gene, in the presence of mimosine.

SH-SY5Y cells cultured with mimosine or DFO for 24 h prior to transfection with pCSHMT-luc displayed no change in luciferase activity relative to SH-SY5Y cells cultured without mimosine (Table IV). Collectively, these data show that folate metabolism is altered by iron chelation in MCF-7 cells but not in SH-SY5Y cells, and the alteration is mediated at least in part by cell-specific changes in cSHMT transcription.

If inhibition of cSHMT transcription occurs by the same mechanisms that result in the inhibition of DNA replication, then both process should display equal sensitivity to iron chelator concentrations. Fig. 6 demonstrates that mimosine at a concentration of 50 μM inhibits both cSHMT promoter activity and MCF-7 cell proliferation by 50%. DFO at 50 μM also inhibits MCF-7 cell proliferation by 50%, while decreasing cSHMT promoter activity to half maximal values at a concentration of less than 10 μM. Therefore, the mechanisms that inhibit cSHMT gene transcription display similar sensitivity to iron chelators as the mechanisms that inhibit DNA replication in MCF-7 cells.

Mimosine Resistance Ameliorates Repression of cSHMT Transcription—The effect of mimosine on cSHMT promoter activity was quantified in mimosine-resistant MCF-7 cells. If decreased cSHMT expression is required for the inhibition of cell cycle caused by mimosine, then the cSHMT promoter should be less sensitive to mimosine in these mutants. Table IV shows that in four of the five mimosine-resistant colonies studied, the cSHMT promoter activity was inhibited by 350 μM mimosine to a similar degree as wild-type MCF-7 cells. The cSHMT protein levels, in these same clones, were also decreased to a similar degree as observed in wild-type MCF-7 cells following mimosine exposure. These results clearly indicate that mimosine resistance can be achieved without rescuing cSHMT promoter activity.

However, the mutation associated with clone 2A ameliorates repression of cSHMT transcription in the presence of mimosine (Table V). Clone 2A displays the highest level of cSHMT promoter activity in the presence of mimosine (Table V), and mimosine was not able to effectively deplete cSHMT protein levels in this clone (Fig. 7). In addition, the basal cSHMT promoter activity in the absence of mimosine is elevated 2-fold in this clone. These results indicate that the mutation in clone 2A renders mimosine ineffective in blocking cell cycle, decreasing cSHMT protein levels and inhibiting cSHMT promoter activity. Therefore, the results obtained from clone 2A indicate that rescue of cSHMT promoter activity is associated with mimosine resistance. These results show that mutations that confer mimosine resistance can also ameliorate the inhibitory effect of mimosine on cSHMT promoter activity, suggesting a common regulatory mechanism for these processes.

**DISCUSSION**

The results of this study are the first to present a biochemical mechanism that accounts for animal and clinical studies that demonstrate that iron deficiency modifies folate metabolism in a tissue-specific manner (5, 6). Using iron chelators to simulate iron deficiency, we show that the effect of iron on folate metabolism is mediated, at least in part, by changes in cSHMT expression. The iron chelator DFO induces the same effects on cSHMT expression as mimosine, and the chemical compositions of mimosine and DFO are highly dissimilar. This suggests that it is the capacity of mimosine to chelate iron that modifies folate metabolism and cSHMT expression. Although previous studies have demonstrated that mimosine binds the SHMT...
The cSHMT promoter activity was determined by transfecting the pcSHMT-luc and pcSHMT-SV40-luc constructs into mammalian cells cultured in the presence of 350 μM mimosine, 150 μM DFO, or 350 μM tyrosine, using a dual luciferase gene reporter assay as described under “Experimental Procedures.” Cells were pretreated with these inhibitors for 20 h prior to transfection. Following transfection, cells were cultured for an additional 48 h in the same medium prior to determining luciferase activity. Data are presented as pGL3-basic firefly luciferase activity relative to pRL-CMV Renilla luciferase activity. All reported luciferase activity values represent the mean of at least five measurements ± S.E.

![Effect of iron chelators on cSHMT promoter in MCF-7 and SH-SY5Y cells](http://www.jbc.org/)

**FIG. 6.** Effects of mimosine and DFO on cell proliferation and pcSHMT-luc activity in MCF-7 cells. A, MCF-7 cells were cultured for 24 h in the presence of mimosine (squares) and DFO (triangles). Following this pretreatment, cells were metabolically labeled with [methyl-3H]thymidine for an additional 24 h, and incorporation into DNA was quantified. All values represent the mean of at least three measurements, and the error bars represent S.E. B, the effect of mimosine (squares) and DFO (triangles) on cSHMT promoter activity was determined using a dual luciferase gene reporter assay as described under “Experimental Procedures.” The pcSHMT-luc construct, which contains the luciferase gene driven by the cSHMT promoter (~1 to ~408), was transfected into MCF-7 cells cultured with αMEM and variable concentrations of DFO or mimosine for 20 h. Cells were cultured for an additional 48 h prior to assaying luciferase activity. Data are presented as relative luminosity of pGL3-basic firefly luciferase to pRL-CMV Renilla luciferase. All reported luciferase activity values represent the mean of at least five measurements, and the error bars represent S.E.

![Effect of iron chelators on cSHMT promoter activity in mimosine resistant MCF-7 cells](http://www.jbc.org/)

**FIG. 7.** Effect of mimosine on cSHMT enzyme levels in a mimosine-resistant MCF-7 cell line. The cSHMT protein levels were determined in a mimosine-resistant cell line 2A cultured in the absence (lane A) and presence (lane B) of 350 μM mimosine for 24 h. Cells were harvested, and cSHMT protein levels were determined by Western blots as described under “Experimental Procedures.”

![Folate Metabolism and Iron](http://www.jbc.org/)

### TABLE IV

**Effect of iron chelators on cSHMT promoter in MCF-7 and SH-SY5Y cells**

| Cell line | Vector | Pretreatment/time | Relative luciferase |
|-----------|--------|------------------|--------------------|
| MCF-7     | pcSHMT-luc | αMEM/24 h         | 100.0 ± 14        |
| MCF-7     | pcSHMT-luc | αMEM, mimosine (300 μM)/24 h | 4.3 ± 3       |
| MCF-7     | pcSHMT-luc | αMEM, tyrosine (300 μM)/24 h | 105 ± 50      |
| MCF-7     | pcSHMT-luc | αMEM, DFO (150 μM)/24 h | 3.6 ± 2        |
| SH-SY5Y   | pcSHMT-luc | αMEM/24 h         | 100.0 ± 25.0      |
| SHSY-5Y   | pcSHMT-luc | αMEM/24 h         | 144.0 ± 28       |
| MCF-7     | pcSHMT-SV40-luc | αMEM/24 h | 100.0 ± 14       |
| MCF-7     | pcSHMT-SV40-luc | αMEM, mimosine (300 μM)/24 h | 25 ± 8       |

### TABLE V

**Effect of iron chelators on cSHMT promoter activity in mimosine resistant MCF-7 cells**

| Cell type | Luciferase activity without mimosine | Luciferase activity with mimosine |
|-----------|-------------------------------------|----------------------------------|
| MCF-7     | 1.0 (100%)                          | 0.05 (100%)                      |
| 2A        | 1.9± (190%) b                       | 0.24± (480%)                     |
| 3B        | 1.2 (120%)                          | 0.11± (220%)                     |
| 4B        | 1.1 (110%)                          | 0.08± (160%)                     |
| 4A        | 0.8 (80%)                           | 0.06 (120%)                      |
| 5B        | 0.4± (40%) a                        | 0.06 (120%)                      |

a represents two-tailed p values ≤ 0.005.
b + represents two-tailed p values ≤ 0.002.

induce clinical symptoms of folate deficiency and even death in both animals and humans (30). The accumulation of 5-methylTHF that is associated with a loss in cSHMT protein supports the notion that cSHMT can regulate the homocysteine remethylation cycle (16). Additionally, inhibition of cSHMT activity by expressing cSHMT antisense constructs also results in the accumulation of 5-methylTHF in MCF-7 cells.2 Therefore, the conversion of glycine to serine catalyzed by cSHMT regulates the flux of one-carbon units through the homocysteine remethylation pathway, in MCF-7 cells. In addition, our data suggest that iron chelators indirectly influence the regulation of homocysteine remethylation.

Although other studies have demonstrated that mimosine and hydroxyurea decrease dATP and dGTP pools in other mammalian cell lines (9), this effect is probably not directly
responsible for the effectiveness of mimosine as an inhibitor of DNA replication. We have also determined that mimosine does not alter dNTP pools in MCF-7 cells, and the results of this study demonstrate that mimosine does not inhibit cell cycle in SH-SY5Y neuroblastoma. Therefore, although mimosine can inhibit both dNTP synthesis and folate metabolism in some cell lines, there is no consistent correlation between these effects and the inhibition of DNA replication. Finally, analyses of mimosine-resistant MCF-7 cell lines demonstrate that the inhibitory effect of mimosine on the cell cycle can be rescued without rescuing inhibition of cSHMT promoter activity. This further demonstrates that the effects of mimosine on folate metabolism do not directly influence DNA replication.

Because the effects of mimosine on DNA replication do not arise from alterations in dNTP pools secondary to modified RNR or SHMT activity, alternative mechanisms must be considered. The results of this study strongly indicate that the mechanisms that lead to inhibition of DNA replication and inhibition of cSHMT promoter activity are related. Both events show a similar sensitivity to iron chelator concentrations, and both events are rescued in a mimosine-resistant cell line. These results suggest that a cis-acting silencer element is present within the cSHMT proximal promoter that responds to iron chelation and is undoubtedly present in other genes. Therefore, genes regulated by this consensus sequence would have altered transcription rates in the presence of mimosine.

The common effect of mimosine on inhibition of cSHMT transcription and cell cycle specificity for iron chelators. Aphidicolin, a DNA polymerase inhibitor that is used to block cell cycle progression at the G1/S boundary, does not deplete cSHMT enzyme levels, nor does it inhibit cSHMT promoter activity as seen for mimosine. Hydroxyurea, an agent that also blocks the cell cycle at the G1/S boundary, has also been shown to depress deoxynucleotidyl pools (31), but it does not influence cSHMT protein levels. Therefore, the effect of iron chelators on cSHMT expression in MCF-7 cells appears to be unique. The identification of this mimosine-sensitive transcription element and the genes that it regulates will likely enhance not only our understanding of the mechanism through which mimosine inhibits cell proliferation but also our understanding of the role of iron in influencing folate metabolism.

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