Modulation of transferrin receptor mRNA by transferrin–gallium in human myeloid HL60 and lymphoid CCRF-CEM leukaemic cells

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Gallium binds to the iron transport protein transferrin (Tf), is incorporated into cells through transferrin receptors (TfR) and inhibits iron-dependent DNA synthesis. Since cellular TfR expression is tightly regulated by the availability of iron, we investigated the effects of transferrin–gallium (Tf–Ga) on TfR mRNA levels in myeloid HL60 and lymphoid CCRF-CEM cells. In HL60 cells, Tf–Ga increased TfR mRNA levels in a dose-dependent fashion. This increase in TfR mRNA was blocked by Tf–Fe and by cycloheximide. Analysis of the rate of mRNA decay in the presence of actinomycin D revealed that the half-life of TfR mRNA was increased in HL60 cells incubated with Tf–Ga. The rate of transcription of TfR mRNA was not increased by Tf–Ga. In contrast with HL60 cells, CCRF-CEM cells displayed a decrease in the level of TfR mRNA after incubation

with Tf–Ga. Tf–Ga inhibited iron uptake in both HL60 and CCRF-CEM cells but increased the level of TfR mRNA only in HL60 cells, suggesting that the Tf–Ga induction of TfR mRNA was not solely due to inhibition of cellular iron uptake. At growth-inhibitory concentrations, Tf–Ga increased the TfR mRNA level in HL60 cells but decreased it in CCRF-CEM cells. Our studies suggest that in HL60 cells, gallium regulates TfR expression at the post-transcriptional level by mechanisms which require *de novo* protein synthesis and involve interaction with iron. The divergent effects of Tf–Ga on TfR mRNA in myeloid HL60 and lymphoid CCRF-CEM cells suggest that differences exist in the regulation of TfR expression between these two cell types.

INTRODUCTION

Iron is an essential component of critical enzymes involved in the maintenance of cellular viability and DNA synthesis. In the circulation, iron is bound to transferrin (Tf) and its uptake by cells is mediated by cell-surface transferrin receptors (TfRs) [1]. The amount of TfR varies in different tissues and is related, in part, to the proliferative state of the cells and their requirements for iron. For example, large densities of TfR are found on erythroid cells (iron required for haemoglobin production) as well as on proliferating, non-erythroid cells [1,2]. The increase in the level of TfR seen as cells enter S phase may, in part, reflect cellular requirements for iron needed for the activity of ribonucleotide reductase, the iron-dependent enzyme responsible for the synthesis of deoxyribonucleotides [3]. The total number of TfRs decline when cells are induced to differentiate and cease to proliferate [2,4]. In addition to the changes in TfRs associated with cellular proliferation, TfR expression is closely regulated by the availability of iron to the cell. Under conditions of iron deprivation stabilization of TfR mRNA occurs [5,6], resulting in an increase in TfR synthesis [7,8].

Gallium, a group IIIA metal has significant clinical activity in the treatment of hypercalcaemia, lymphoma and bladder cancer [9–11]. In previous studies, we have investigated the interaction of gallium with different leukaemic cell lines *in vitro* and have shown that gallium inhibits cell growth by mechanisms which involve blockade of cellular iron incorporation and inhibition of ribonucleotide reductase [12,13]. Gallium shares several properties with iron with respect to Tf binding [14] and cellular uptake by TfR-dependent and -independent mechanisms [15,16]. Since no information exists regarding its effect on the regulation of

TfR expression at the molecular level, we have compared the effects of Tf–Ga on TfR mRNA in human myeloid HL60 and lymphoid CCRF-CEM cells.

MATERIALS AND METHODS

Materials

Human apotransferrin (apoTf), FeCl₃, actinomycin D, cycloheximide, phenylmethanesulphonyl fluoride, dithiothreitol (DTT) and spermidine were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Gallium nitrate was purchased from Alfa Products (Danvers, MA, U.S.A.). Recombinant human zinc insulin was purchased from Calbiochem (La Jolla, CA, U.S.A.). [³²P]dCTP, [³²P]UTP and ⁵⁹FeCl₃ were obtained from New England Nuclear (Boston, MA, U.S.A.) and Amersham (Arlington Heights, IL, U.S.A.). Tf–Fe and ⁵⁹Fe–Tf and Tf–Ga were prepared as described previously [17]. Human TfR cDNA (pcDTR1) was obtained from American Type Culture Collection (Rockville, MD, U.S.A.). The pcDTR1 was amplified and purified by PZ523 spin column (5 Prime – 3 Prime Inc., Boulder, CO, U.S.A.) as suggested by the manufacturer. TfR cDNA insert (4.9 kb) was excised with *Bam*HI and isolated by electroelution. RNA levels were normalized by using a human β -actin cDNA probe (Clontech Laboratories Inc., Palo Alto, CA, U.S.A.). cDNA probes were labelled with [³²P]dCTP (specific radioactivity 3000 Ci/mmol) using a random primer extension labelling system according to the manufacturer's instructions (NEN Dupont, Boston, MA, U.S.A.). pBR322 DNA, RNAase inhibitor and ribonucleotides were purchased from Promega (Madison, WI, U.S.A.).

Abbreviations used: Tf, transferrin; Tf–Fe, transferrin–iron; Tf–Ga, transferrin–gallium; apoTf, apotransferrin; TfR, transferrin receptor; DTT, dithiothreitol; SSC, 0.15 M NaCl/0.015 M sodium citrate; IRE, iron-responsive element.

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Cells and cell culture conditions

Human promyelocytic leukaemic HL60 and lymphoid CCRF-CEM cells were obtained from American Type Culture Collection (Rockville, MD, U.S.A.). HL60 cells were maintained in RPMI 1640 medium containing 10% (v/v) fetal-calf serum containing 200 units/ml of penicillin, 0.2 mg/ml of streptomycin and 50 µg/ml gentamicin. CCRF-CEM cells were maintained in the same medium but without antibiotics. The presence or absence of these antibiotics in the culture medium did not influence the results obtained on Northern-blot analysis. Cells were incubated at 37 °C in an atmosphere of 6% CO₂. For experiments, cells were plated at 10⁶ cells/ml in serum-free media (RPMI 1640) containing 5 µg/ml human insulin. ApoTf or Tf-Ga (0–200 µg/ml) was added to cells and cells were then incubated at 37 °C for 24–48 h. Cell viability was determined by Trypan Blue exclusion. RNA was isolated from cells as described below.

To determine the effect of inhibition of protein synthesis on TfR mRNA, cells were analysed after a 48-h incubation with 10 µg/ml cycloheximide. To determine the effect of inhibition of transcription on TfR mRNA, cells were first incubated for 48 h with 50 µg/ml of apoTf or Tf-Ga. Actinomycin D (5 µg/ml) was then added to each flask and the incubation continued for an additional 0–6 h, after which cells were harvested and analysed for TfR mRNA.

RNA isolation and Northern-blot analysis

Total RNA was isolated from 2–3.5 × 10⁷ cells by a guanidine–thiocyanate–CsCl method as described [18]. RNA samples (25 µg) were subjected to electrophoresis on a 1% (w/v) agarose gel containing 2.2 M formaldehyde and then transferred to Nytran membranes (Schleicher and Schuell) in 10 × SSC (1 × SSC, 0.15 M NaCl/0.015 M sodium citrate) using a capillary blotting method. The membranes were washed in 5 × SSC, air-dried and baked at 80 °C for 2 h. The membranes were first pre-hybridized for 4 h at 42 °C in 50% formamide/5 × SSPE (1 × SSPE = 150 mM NaCl/10 mM NaH₂PO₄/1 mM EDTA, pH 7.4)/5 × Denhardt's solution, 10% (w/v) dextran sulphate, 1% (w/v) SDS and 200 µg/ml sheared salmon sperm DNA. Membranes were then hybridized overnight in the same solution containing ³²P-labelled TfR cDNA (1 × 10⁶ c.p.m./ml). The membranes were then washed three times in 2 × SSC at room temperature followed by two 30-min washes in 2 × SSC/0.1% SDS at 65 °C. The membranes were exposed to XAR-5 film (Eastman Kodak Co, Rochester, NY, U.S.A.) with intensifying screens at –70 °C for 24 h. For quantification, the band intensities were scanned on an AMBIS Optical Imaging System (AMBIS, San Diego, CA, U.S.A.).

Nuclear run-on assay

The effects of Tf-Ga on the rate of transcription of TfR mRNA in isolated nuclei were examined using the method described by Marzluff and Huang [19]. HL60 cells were incubated with 50 µg/ml apoTf or Tf-Ga for 48 h in serum-free medium containing 5 µg/ml of recombinant human insulin. Cells (10⁸) were disrupted by 10–15 strokes in a Dounce homogenizer in 3 ml of 10 mM Tris/HCl buffer, pH 8, containing 0.32 M sucrose, 3 mM CaCl₂, 2 mM magnesium acetate, 1 mM EDTA, 1 mM DTT, 0.1 mM phenylmethanesulphonyl fluoride, 1 mM spermidine and 0.1% Triton X-100. Buffer A (10 mM Tris/HCl buffer, pH 8.0, containing 2 M sucrose, 5 mM magnesium acetate, 0.1 mM EDTA and 1 mM DTT) (3 ml) was added to the

lysate. The sample was then layered over a 3-ml cushion of buffer A and centrifuged at 25000 rev./min in a TH641 Sorvall rotor at 4 °C for 45 min. The pelleted nuclei were resuspended at a concentration of 4 × 10⁸ nuclei/ml in 50 mM Tris/HCl buffer, pH 8, containing 5 mM magnesium acetate, 0.1 mM EDTA, 5 mM DTT and 25% (v/v) glycerol.

Nuclear transcription assays were performed with 3 × 10⁷ nuclei in a volume of 200 µl containing 0.5 mM each of ATP, CTP and GTP, 20 µM [³²P]UTP (1000 µCi/ml), 5 µl RNasin, 0.25 mM S-adenosylmethionine, 0.12 M KCl, 5 mM magnesium acetate, 12.5% (v/v) glycerol, 0.05 mM EDTA, 2.5 mM DTT and 25 mM Tris/HCl, pH 8. The reaction was continued for 45 min at 26 °C and was terminated by the addition of 2 ml of 10 mM EDTA/1% (w/v) SDS. The ³²P-labelled RNA was isolated as described [19] and used for hybridization. Plasmid DNA pcDTR1 (TfR cDNA) or pBR322 (control) were linearized and were immobilized on Nytran membranes using a slot blot apparatus. The membranes were then pre-hybridized for 4 h in the solution used for Northern blotting supplemented with 50 µg/ml tRNA. ³²P-labelled mRNAs (4.5 × 10⁶ c.p.m.) obtained from nuclear transcription were hybridized to membranes at 42 °C for 4 days. After hybridization, the membranes were washed sequentially at 65 °C for 30 min in 2 × SSC containing 0.1% SDS and 0.2 × SSC containing 0.5% SDS. This was followed by treatment of the membranes for 30 min at 37 °C with RNAase A (10 µg/ml) and RNAase T1 (100 units/ml) in 0.3 M NaCl/2 mM EDTA/10 mM Tris/HCl buffer, pH 7.5. The membranes were then finally washed in the same buffer without RNAase and autoradiography of the membranes was performed at –70 °C for 5 days using XAR-5 film (Eastman Kodak Co, Rochester, NY, U.S.A.) with intensifying screens.

⁵⁹Fe uptake studies

To determine the effect of Tf-Ga on ⁵⁹Fe uptake by cells, HL60 and CCRF-CEM cells were plated in serum-free medium (containing 5 µg/ml human insulin) in 1-ml well capacity multiwell plates (10⁶ cells/well) in the presence of 0, 50 or 100 µg/ml Tf-Ga. ⁵⁹Fe-Tf (38000 c.p.m.) ⁵⁹Fe-Tf, equivalent to 3.1 ng of Fe and 2.1 µg of Tf) was added to each well. After 6, 24 and 48 h of incubation at 37 °C, cells were harvested, washed with PBS and the radioactivity in the cell pellet was counted to determine ⁵⁹Fe uptake by cells.

RESULTS

Induction of TfR mRNA in HL60 cells by Tf-Ga

As shown in Figure 1, exposure of HL60 cells to 25–200 µg/ml of Tf-Ga resulted in a progressive increase in TfR mRNA when compared with cells incubated with corresponding concentrations of apoTf alone. At a concentration of 50 µg/ml, Tf-Ga induced an approx. 4-fold increase in TfR mRNA levels (Figure 1, lane 3 versus lane 4). At 100 µg/ml Tf-Ga, there was a 9-fold increase in TfR transcripts, which plateaued at 200 µg/ml (Figure 1, lanes 5 and 7 versus lanes 6 and 8). During this period (48 h) the growth of HL60 cells exposed to 200 µg/ml of Tf-Ga was approx. 50% of control levels, while 25 and 50 µg/ml of Tf-Ga had no significant effect on cellular proliferation. Therefore, subsequent studies were performed with cells exposed to 50 µg/ml of apoTf or Tf-Ga.

Hybridization intensities of β-actin mRNA were not affected with increasing apoTf or Tf-Ga concentrations (Figure 1, lanes 1–8) so this probe was used as a positive control to normalize loading of RNA for Northern-blot analysis.

Figure 1 Induction of TfR mRNA in HL60 cells by Tf-Ga

Cells were incubated for 48 h with increasing concentrations of apoTf or Tf-Ga. Total RNA was isolated and analysed for TfR mRNA by Northern blot. Lanes 1, 3, 5 and 7 represent cells incubated with 25, 50, 100 or 200 µg/ml apoTf. Lanes 2, 4, 6 and 8 represent cells incubated with corresponding concentrations of Tf-Ga. Each lane was loaded with 25 µg of RNA. The same filter was hybridized with the β -actin cDNA probe to normalize RNA loading.

Inhibition of Tf-Ga-Induced TfR mRNA Increase by Tf-Fe

Because of the similarity between gallium and iron with respect to Tf binding and cellular uptake, we investigated whether the Tf-Ga-mediated increase in TfR mRNA was influenced by Tf-Fe. Increasing concentrations of Tf-Fe were added to HL60 cells incubated in medium containing Tf-Ga. Consistent with previous reports [5,6,20], Tf-Fe alone markedly decreased the baseline expression of TfR (Figure 2a, lanes 5 and 6). The presence of equimolar concentrations of Tf-Fe (50 µg/ml) during incubation of cells with Tf-Ga completely blocked the gallium-induced increase in TfR mRNA (Figure 2a, lanes 7 and 8, and Figure 2b, lanes 3 and 4). However, this inhibition was reversed as the concentration of Tf-Fe was reduced to 12.5 µg/ml (Figure 2b, lanes 7 and 8). These results suggest that the increase in TfR mRNA induced by Tf-Ga is mediated through interaction with an iron-dependent process.

Effects of cycloheximide and actinomycin D on TfR mRNA in HL60 cells

Because maximal induction of TfR mRNA was seen after 48 h of incubation with Tf-Ga, HL60 cells were incubated for this period of time with 10 µg/ml of cycloheximide and then analysed by Northern blot. No differences in cell viability were observed between apoTf- and Tf-Ga-treated cells after exposure to cycloheximide. In the presence of cycloheximide, the gallium-induced increase in TfR mRNA levels was blocked (Figure 3), suggesting that a labile protein was required for this induction.

The gallium-induced increase in TfR mRNA levels in HL60 cells could result from an increase in the transcription or a decrease in the degradation of mRNA. To determine the mechanism involved, mRNA transcription was blocked with actinomycin D after cells had been incubated with apoTf or Tf-Ga for 48 h. Changes in TfR mRNA levels in both cell groups were then compared over time. As shown in Figure 4, the rate of decay of TfR mRNA in Tf-Ga-treated cells was slower than that of apoTf-treated cells. Over the 0–6 h time points TfR mRNA levels in apoTf cells steadily diminished. In contrast, no significant decrease in TfR mRNA levels was seen between the 0 and 2 h

Figure 2 Tf-Fe prevents the Tf-Ga-induced increase in TfR mRNA

(a) Northern blot of TfR mRNA in absence or presence of Tf-Fe. Lanes 1 and 2, apoTf 50 µg/ml; lanes 3 and 4, Tf-Ga 50 µg/ml; lanes 5 and 6, Tf-Fe 50 µg/ml; lanes 7 and 8, HL60 cells incubated simultaneously with 50 µg/ml Tf-Ga and 50 µg/ml Tf-Fe. (b) Northern blot of TfR mRNA from HL60 cells incubated with Tf-Ga and decreasing concentrations of Tf-Fe. Lanes 1 and 2, Tf-Ga 50 µg/ml alone; lanes 3 and 4, Tf-Ga 50 µg/ml and Tf-Fe 50 µg/ml; lanes 5 and 6, Tf-Ga 50 µg/ml and Tf-Fe 25 µg/ml; lanes 7 and 8, Tf-Ga 50 µg/ml and Tf-Fe 12.5 µg/ml.

time points in the Tf-Ga-treated cells, indicating an increase in the stability of TfR mRNA in these cells. Based on densitometry of the Northern blot, it was estimated that the half-life of TfR mRNA was 2.9 and 4.5 h respectively in apoTf- and Tf-Ga-treated cells. The half-life of TfR mRNA in apoTf-treated cells in our studies is consistent with that reported by others [20].

Effect of Tf-Ga on the rate of transcription of TfR mRNA in HL60 cells

To confirm that the increase in TfR mRNA seen in HL60 cells after exposure to Tf-Ga was not the result of an increase in the rate of TfR mRNA transcription, nuclear run-on transcription assays were performed on nuclei isolated from Tf-Ga- and apoTf-treated cells. Plasmid pBR322 DNA was used as a control to monitor for non-specific hybridization of 32 P-labelled mRNA to Nytran membranes [20]. As shown in Figure 5, no major differences were noted in the rate of TfR mRNA transcription between cells incubated with Tf-Ga and cells incubated with apoTf. These data further support our conclusions that the Tf-Ga-induced increase in TfR mRNA levels in HL60 cells was due to regulation at the post-transcriptional level.

Figure 3 Effect of cycloheximide on TfR mRNA

HL60 cells were incubated for 48 h with 50 μ g/ml Tf-Ga or 50 μ g/ml apoTf in the presence or absence of 10 μ g/ml of cycloheximide (cyclohex).

Figure 4 Tf-Ga increases the half-life of TfR mRNA

HL60 cells were incubated for 48 h with 50 μ g/ml of apoTf or Tf-Ga. Actinomycin D was then added to cells (0 h) and the incubation continued for an additional 6 h. RNA was isolated from cells at the time points shown and analysed for TfR mRNA.

Effects of Tf-Ga on TfR mRNA in CCRF-CEM cells

To investigate whether Tf-Ga affects TfR expression similarly in cells of lymphoid origin, CCRF-CEM cells were analysed after incubation with increasing concentrations of Tf-Ga. TfR mRNA levels decreased in a dose-dependent manner effective at 50 μ g/ml (Figure 6, compare lanes 3 and 4). Higher concentrations of Tf-Ga caused a further decrease in TfR mRNA (Figure 6, compare lane 5 with lane 6, and lane 7 with lane 8). These results are in contrast with the effects of Tf-Ga in HL60 cells, indicating that gallium has opposing effects on the expression of TfR in these two cell lines.

Effect of Tf-Ga on ^{59}Fe uptake by HL60 and CCRF-CEM cells

Since our earlier studies have shown that Tf-Ga inhibits the uptake of ^{59}Fe by HL60 cells, we examined whether HL60 cells differed from CCRF-CEM cells with respect to Tf-Ga-induced effects on iron uptake. As shown in Table 1, Tf-Ga inhibited iron

Figure 5 Transcription of TfR mRNA in HL60 cells exposed to Tf-Ga or apoTf

Nuclei were isolated from HL60 cells after a 48-h incubation with 50 μ g/ml of apoTf or Tf-Ga. Nuclear run-on transcription assays were performed as described in the Materials and methods section. ^{32}P -labelled mRNA was hybridized to TfR cDNA or pBR322 DNA (control) immobilized on Nytran membranes.

Figure 6 Effect of Tf-Ga on TfR mRNA in CCRF-CEM cells

Cells were incubated with 25, 50, 100 and 200 μ g/ml of apoTf (lanes 1, 3, 5 and 7) or Tf-Ga (lanes 2, 4, 6 and 8). Each lane was loaded with 25 μ g of RNA. The same filter was hybridized with the β -actin cDNA probe to normalize RNA loading.

Table 1 Inhibition of cellular uptake of ^{59}Fe by Tf-Ga

Cells (10^6) were plated in 1-ml multi-well plates in serum-free medium in the presence of increasing concentrations of Tf-Ga. ^{59}Fe -Tf was added at the start of incubation. At the specified time points, cells were harvested, washed, and ^{59}Fe content (c.p.m.) in the cell pellet determined. Values represent the mean $\pm$ S.E.M. of an experiment performed in triplicate. Values given in parentheses are percentages of the control levels.

Time (h)	Tf-Ga (μ g/ml)	^{59}Fe uptake (c.p.m.)	
		HL60	CCRF-CEM
6	0	8077 $\pm$ 110 (100)	4491 $\pm$ 376 (100)
	50	1448 $\pm$ 13 (18)	1359 $\pm$ 29 (30)
	100	915 $\pm$ 8 (11)	846 $\pm$ 11 (19)
24	0	22541 $\pm$ 399 (100)	15363 $\pm$ 649 (100)
	50	4802 $\pm$ 46 (21)	4493 $\pm$ 61 (29)
	100	2435 $\pm$ 36 (11)	2029 $\pm$ 25 (13)

uptake in both cell lines in a comparable manner. Significant inhibition of iron uptake was seen at the time points shown, including after 48 h of incubation (results not shown). Hence, the opposite effects of Tf-Ga on TfR mRNA levels in HL60 cells and CCRF-CEM cells does not appear to be the result of differences in iron uptake between the two cell lines.

DISCUSSION

Previously, we have demonstrated that gallium interferes with Tf-dependent and Tf-independent iron transport across cell membranes [17,21], blocks the incorporation of iron into ferritin and haemoglobin [17,22], and inhibits iron-dependent DNA synthesis [12,13]. While we have found that incubation of HL60 cells with Tf-Ga results in an increase in cell-surface TfR on these cells [17], it has not been determined whether this increase is due to gallium-induced changes at the mRNA level. Because TfR expression on cells appears to be closely linked to the rate of cellular proliferation and DNA synthesis, we have now focused on comparing the effects of gallium on TfR mRNA in myeloid HL60 and lymphoid CCRF-CEM cells under conditions of normal proliferation and growth arrest.

In the present study, we have shown that gallium delivered to HL60 cells as Tf-Ga induced an increase in TfR mRNA. This increase in TfR mRNA was abrogated in a dose-dependent manner by simultaneous exposure of cells to Tf-Fe, thus indicating that gallium influences TfR mRNA through interaction with iron/iron-dependent processes. It is known that TfR mRNA have, at their 3' untranslated region, conserved sequences termed iron-responsive elements (IREs) which are capable of binding to a regulatory cytoplasmic iron-sulphur protein with aconitase activity (termed the IRE-binding protein) [23,24]. Under conditions of iron deprivation, increased binding of this protein to TfR mRNA IRE occurs, resulting in a prolongation of half-life of the TfR mRNA and increased TfR synthesis [5,6]. That such a mechanism may be involved in the gallium-induced increase in TfR mRNA in HL60 cells is suggested by the following: (1) gallium inhibited iron uptake by HL60 cells, potentially causing cellular iron deprivation; (2) the gallium-induced increase in TfR mRNA levels was blocked by cycloheximide, indicating that this effect was dependent on *de novo* protein synthesis; (3) the half-life of TfR mRNA in gallium-treated cells was greater than that of control cells, a situation which would lead to increased TfR synthesis; and (4) nuclear transcription assays revealed that Tf-Ga did not increase the rate of transcription of TfR mRNA over that of control cells, thus suggesting that the Tf-Ga-induced increase in TfR mRNA is the result of an increase in TfR mRNA stability. Studies are planned to determine whether the protein inhibited by cycloheximide is an mRNA IRE-binding protein and whether gallium increases the level of TfR mRNA by interacting directly with this protein.

The effects of Tf-Ga on TfR mRNA in lymphoid CCRF-CEM cells appear to be complex and are, at present, more difficult to interpret. As with HL60 cells, the cellular uptake of iron by CCRF-CEM cells was blocked by Tf-Ga. In contrast with HL60 cells, however, CCRF-CEM cells did not increase their TfR mRNA levels after incubation with Tf-Ga. Since a decrease in the rate of cellular proliferation in non-haemoglobin-producing cells is usually associated with a corresponding reduction in the expression of TfR [4,25–27], it may at first, appear that the decrease in TfR mRNA in CCRF-CEM cells was

primarily the result of an inhibition of cellular proliferation by Tf-Ga. However, concentrations of Tf-Ga which inhibited the growth of both HL60 and CCRF-CEM cells had divergent effects on their TfR mRNA and induced an increase in levels of TfR mRNA in HL60 cells and a decrease in levels of TfR mRNA in CCRF-CEM cells. In addition, Tf-Ga concentrations which were not growth-inhibitory also increased TfR mRNA levels in HL60 cells. These studies suggest that the effect of Tf-Ga on TfR mRNA is probably independent of the effect of gallium on cellular proliferation and cannot be explained solely on the basis of a Tf-Ga-induced block in iron uptake. Furthermore, the increase in TfR mRNA levels in HL60 cells may result from a direct interaction of gallium with intracellular iron-dependent processes involved in TfR mRNA regulation. The divergent effects of Tf-Ga on the two cell types suggest that HL60 and CCRF-CEM cells may differ in their intracellular handling of gallium and/or in the mechanisms involved in the regulation of TfR expression. Further studies of the interaction of gallium with cellular iron metabolism may elucidate differences in iron-dependent regulation of gene expression among cells of different lineage.

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REFERENCES

- 1 Seligman, P. A., Klausner, R. D. and Heubers, H. A. (1987) in *The Molecular Basis of Blood Disease* (Stamatoyannopoulos, G., Nienhuis, A. W., Leder, P. and Majerus, P., eds.), pp. 219–244. W. B. Saunders, Philadelphia
- 2 Taetle, R. (1990) *Exp. Hematol.* **18**, 360–365
- 3 Thelander, L. and Reichard, P. (1979) *Annu. Rev. Biochem.* **48**, 133–158
- 4 Chitambar, C. R., Massey, E. J. and Seligman, P. A. (1983) *J. Clin. Invest.* **72**, 1314–1325
- 5 Klausner, R. D. and Harford, J. B. (1989) *Science* **246**, 870–872
- 6 Theil, E. C. (1990) *J. Biol. Chem.* **265**, 4771–4774
- 7 Rao, K. K., Shapiro, D., Mattia, E., Bridges, K. and Klausner, R. (1985) *Mol. Cell. Biol.* **5**, 595–600
- 8 Bridges, K. R. and Cudkovic, A. (1984) *J. Biol. Chem.* **259**, 12970–12977
- 9 Warrell, R. P., Jr. (1991) *Semin. Oncol.* **18**, 26–31
- 10 Warrell, R. P., Jr., Coonley, C. J., Straus, D. J. and Young, C. W. (1983) *Cancer* **51**, 1982–1987
- 11 Seligman, P. A. and Crawford, E. D. (1991) *J. Natl. Cancer Inst.* **83**, 1582–1584
- 12 Chitambar, C. R., Mattheus, W. G., Antholine, W. E., Graff, K. and O'Brien, W. J. (1988) *Blood* **72**, 1930–1936
- 13 Chitambar, C. R., Narasimhan, J., Guy, J., Sem, D. S. and O'Brien, W. J. (1991) *Cancer Res.* **51**, 6199–6201
- 14 Harris, W. R. and Pecoraro, V. L. (1983) *Biochemistry* **22**, 292–299
- 15 Larson, S. M., Rasey, J. S., Allen, D. R., Nelson, N. J., Grunbaum, Z., Harp, G. D. and Williams, D. L. (1980) *J. Natl. Cancer Inst.* **64**, 41–53
- 16 Chitambar, C. R. and Zivkovic, Z. (1987) *Cancer Res.* **47**, 3929–3934
- 17 Chitambar, C. R. and Seligman, P. A. (1986) *J. Clin. Invest.* **78**, 1538–1546
- 18 Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. and Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299
- 19 Marzluff, W. F. and Huang, R. C. C. (1984) in *Transcription and Translation. A Practical Approach* (Hames, B. D. and Higgins, S. J., eds.), pp. 89–129. IRL Press, Oxford
- 20 Taetle, R., Ralph, S., Smedsrud, S. and Trowbridge, I. (1987) *Blood* **70**, 852–859
- 21 Chitambar, C. R. and Sax, D. (1992) *Blood* **80**, 505–511
- 22 Chitambar, C. R. and Zivkovic, Z. (1987) *Blood* **69**, 144–149
- 23 Kaptain, S., Downey, W. E., Tang, C., Philpott, C., Haile, D., Orloff, D. G., Harford, J., Rouault, T. and Klausner, R. D. (1991) *Proc. Natl. Acad. Sci. U.S.A.* **88**, 10109–10113
- 24 Haile, D. J., Rouault, T. A., Tang, C. K., Chin, J., Harford, J. B. and Klausner, R. D. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 7536–7540
- 25 Trowbridge, I. S. and Omary, M. B. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3039–3043
- 26 Larrick, J. W. and Cresswell, P. (1979) *J. Supramol. Struct.* **11**, 579–586
- 27 Forsbeck, K. and Nilsson, K. (1985) *Scand. J. Haematol.* **35**, 145–154