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Increased glutathione *S*-transferase P1-1 expression by mRNA stabilization in hemin-induced differentiation of K562 cells

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Abstract

GSTP1-1 gene expression mechanisms were investigated in hemin-induced erythroid differentiation of K562 cells. Hemoglobin production during differentiation was followed by a significant increase in GSTP1-1 mRNA (1.7-fold, $P < 0.01$) and protein (1.2-fold, $P < 0.01$) after 4 days of induction. This increase in mRNA production was not due to transcriptional up-regulation by GATA-1 previously shown to regulate GSTP1-1 during erythroid and megakaryocytic differentiation. Moreover, a drastic decrease in differentiation-specific GATA-1 mRNA expression was correlated to a reduction in GATA-1 promoter binding activity. Neither AP-1 nor NF-κB transcription factor binding activities could provide an explanation to the GSTP1-1 mRNA overexpression in hemin-treated cells. GSTP1-1 mRNA stability analysis using actinomycin D as an inhibitor of mRNA neosynthesis showed that mRNA half-life was doubled in hemin-induced erythroid differentiation of K562 cells. These results allow us to add stabilization of GSTP1-1 mRNA as a novel regulatory mechanism during hemin-mediated differentiation of K562 cells.

Keywords: K562; Erythroid differentiation; Hemin; GSTP1-1; GATA-1; mRNA stability

1. Introduction

Glutathione *S*-transferases (GSTs, EC.2.5.1.18) are a family of multifunctional enzymes that play a crucial role in cellular detoxification by catalyzing the nucleophilic addition of glutathione to a broad range of endogenous and exogenous hydrophobic electophilic compounds [1]. GSTs are dimeric proteins which are classified into seven cytosolic classes (α, μ, π, θ, ω, σ, ξ), one mitochondrial family (κ) and one microsomal membrane-bound family [2,3].

The human pi class of GST, GSTP1-1, is frequently involved in the formation and the progression of tumors [4,5] so that its over-expression is a common feature of many human tumors of a wide range of tissues including, blood, stomach, brain and colon [6–10] and that a high level of GSTP1-1 expression is a bad prognostic for patient survival [11]. In addition, GSTP1-1 is involved in resistance against antineoplastic drugs [12–14] and inhibits apoptosis by binding to the Jun N-terminal Kinase (JNK) [15]. However, in normal cells, increased GSTP1-1 expression is frequently associated with conditions of physiological cellular stress [1].

Concerning the molecular mechanisms underlying transcriptional regulation of the GSTP1-1 gene expression we and others have characterized structural and functional properties of the human GSTP1-1 promoter. GSTP1-1 gene promoter activity is driven by a TATA-box [16] as well as Spl [16,17], AP-1 [18] and NF-κB [19] binding sites. More recently, we demonstrated that the transcription factor GATA-1 binds to a GATA sequence located in −1211/−1208 of the GSTP1-1 gene promoter. Indeed, we showed a correlation between GATA-1 binding activity as well as GSTP1-1 mRNA and protein expression during erythroid- and megakaryocytic-induced K562 cell differentiation [20]. K562 cell line is a well-established model system for the study of spontaneous as well as induced differentiation towards erythrocytic, granulocytic, and monocytic lineages [21].

An alternative approach to classical anti-cancer therapy consists in the induction of cell differentiation using low doses of agents devoid of cytotoxic effect [22–25].

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However, it is important to further investigate if this type of approach to treatment may increase cancer cell resistance following cell detoxification by GSTs. In order to determine whether GSTP1-1 gene expression could be linked to induction of erythroid differentiation by pharmacological agents, we investigated the effect of hemin, a canonical erythroid differentiating agent of K562 cells. Hemin exerts a profound effect on cell maturation and promotes foetal hemoglobin synthesis in erythroid progenitors cultured in vitro [26] and serves as a therapeutic agent in human porphyria treatment [27].

Here we establish a connection between hemoglobin production, GSTP1-1 mRNA and protein expression in hemin-treated K562 cells. We studied the response of transcription factors involved in the regulation of GSTP1-1 expression such as GATA-1 [20], NF-κB [19] and AP-1 [18]. Our findings suggest that GSTP1-1 overexpression in hemin-induced differentiation of K562 cells is not primarily due to a transcriptional upregulation but rather to a stabilization of the corresponding mRNA. In conclusion, we demonstrate the existence of post-transcriptional mechanisms regulating GSTP1-1 gene expression during hemin-induced differentiation of K562 cells.

2. Materials and methods

2.1. Cell culture and drug treatments

The human chronic myelogenous leukemia K562 cell line, obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), was grown in RPMI 1640 medium (Bio-Whittaker) supplemented with 10% (v/v) heat-inactivated foetal calf serum (FCS) (Bio-Whittaker) and 1% (v/v) antibiotic–antimycotic (Bio-Whittaker) in 5% CO_2 humidified atmosphere at 37 °C. Erythroid differentiation was induced by the addition of hemin (MP-Biomedicals) at a 30 μM final concentration to the cell suspension at the beginning of the exponential growth phase. TNFα (Sigma) and 12-*O*-tetradecanoyl phorbol 13-acetate (TPA) (MP-Biomedicals) were used at final concentrations of 10 ng/ml and 100 nM, respectively. Cell growth and viability were assessed by trypan blue dye exclusion test. The percentage of cell growth inhibition was then calculated as follows: $[(C_n - C_0) - (T_n - T_0)] \times 100/(C_n - C_0)$ where C_n, C_0, T_n and T_0 represent the number of control (C) or treated (T) cells/ml at days 0 and n, respectively. Determination of erythroid differentiation was realized by the benzidine staining method for the identification of percentage of hemoglobin-positive cells upon induction as previously described [28].

2.2. RNA extraction

Total RNA was extracted from hemin-treated or control K562 cells using Nucleospin RNA II kit (Macherey-Nagel) according to the manufacturer's protocol. To study mRNA stability, cells were first treated or not for 48 h with hemin a 30 μM. Then total RNA was extracted from hemin-treated or control cells after incubation for 24, 36, 48 and 56 h with actinomycin D.

2.3. Analysis of GSTPl-1 gene expression by Northern blot analysis

Northern blot analysis was realized by using a 720-bp fragment of the human GSTP1-1 cDNA (American Type Culture Collection, ATCC) as a probe. Ethidium bromide-stained gel was used as a loading control. Results were acquired on a Cyclone phosphorimager (Perkin-Elmer) and quantified by Kodak ID software (Analis).

2.4. Analysis of GAT A-1 gene expression by PCR

cDNA synthesis was realized using the Superscript™ First-strand Synthesis System for RT-PCR (Invitrogen). Five micrograms of RNA were submitted to reverse transcription (RT) using oligo(dt) primers. The resulting RT products were used as templates for PCR amplification according to the manufacturer's instructions using the Platinium® *Taq* DNA Polymerase High Fidelity and gene-specific primers of GATA-1 (sense: 5′-TCAATTCAGCAGCCTATTCC-3′; antisense: 5′-TTCGAGTCTGAATACCATCC-3′). The amount of cDNA synthesized was evaluated by amplification of the S14 gene (sense: 5′-GGCAGACGAGATGAATCCTC-3′; antisense: 5′-CAGGTCCAGGGGTCTTGGTCC-3′) as a standard. Amplification was performed at 94 °C for 2 min, 60 °C for 1 min and 68 °C for 2 min for 20 cycles. PCR products were separated on a 2% agarose gel.

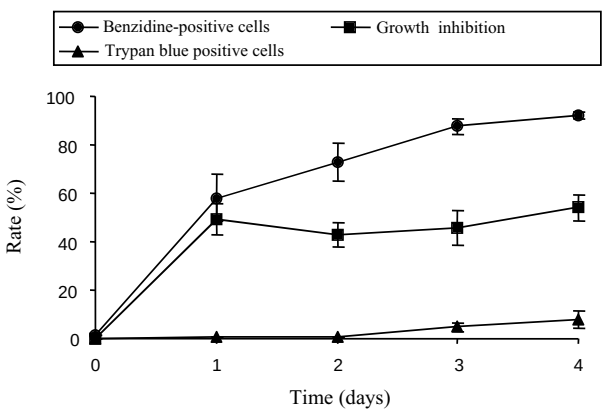

Fig. 1. Effect of hemin on hemoglobin synthesis, viability and growth in K562 cells. Cells were grown in medium alone or treated for various times with hemin (30 μM). Percentage of hemoglobin-producing cells was determined by the benzidine staining method. Effects of hemin-induced differentiation on in vitro growth inhibition and cellular viability are indicated. These data are the means (±S.D.) of three independent experiments.

2.5. Preparation of nuclear and cytosolic extracts from K562 cells

After treatment, cells were harvested and washed. Extracts were prepared from K562 cells according to Muller et al. [29]. Protein content was determined for each sample using the Bradford assay (Bio-Rad).

2.6. SDS–polyacrylamide gel electrophoresis and western blotting

Five micrograms of proteins from cytosolic extracts were resolved using SDS–PAGE and western blots were realized using a polyclonal GSTP1-1 antibody (Clontech) or a monoclonal mouse anti-β-actin (Sigma) primary antibody as loading control. Results were acquired by a Kodak

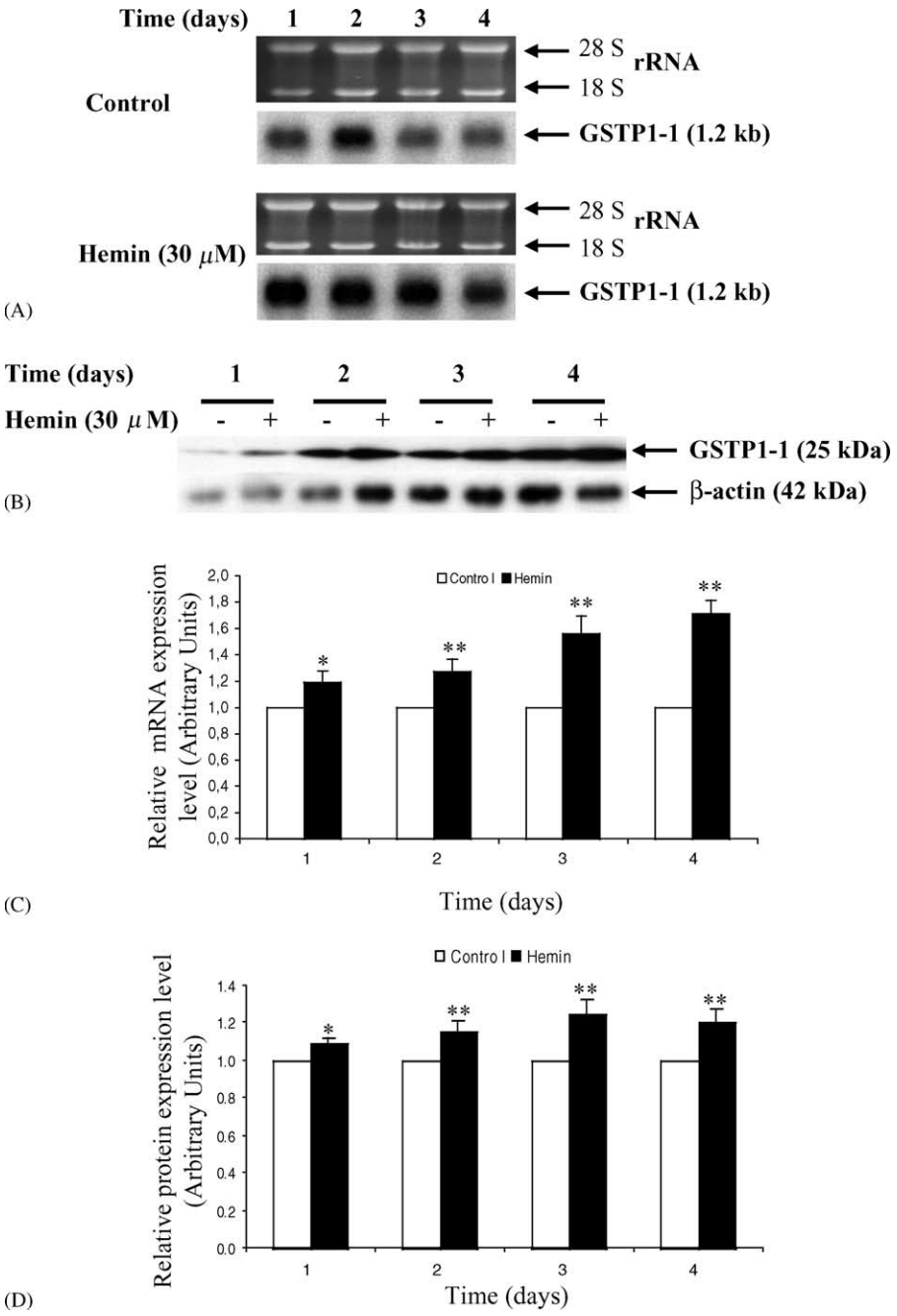

Fig. 2. Effect of hemin-induced erythroid differentiation on GSTP1-1 mRNA (A) and protein (B) expression. (C) Northern blots were quantified by phosphorimaging and results were expressed as a ratio between GSTP1-1 and rRNA 18S signals. (D) Western blots results were expressed as a ratio between GSTP1-1 and β-actin hybridization signals. Data are mean ± S.E.M. of three independent experiments. (*) and (**) indicate $P < 0.05$ and $P < 0.01$ compared to control, respectively.

440 chemoluminescence reader and quantified by Kodak 1D software (Analis).

2.7. Electrophoretic mobility shift assay (EMSA)

Oligonucleotides (Eurogentec) corresponding to relevant GSTP1-1 promoter sequences were used as probes: probe GATA, nucleotides −1219 to −1200, 5′-AGCTAAGG**GATA**CTGGGCTT-3′; probe NF-κB, nucleotides −327 to −305, 5′-TCTTA**GGGAATTTCC**CCCCGCGA-3′; probe AP-1, nucleotides −73 to −54, 5′-GCCG-**TGACTCA**GCACTGGGG-3′. Gelshift analysis were realized as described by Borde-Chiche et al. [18]. For supershift experiments, nuclear proteins were preincubated with anti-GATA-1 (Active Motif), anti-NF-κB p50 (Santa Cruz) or anti-cjun (Santa Cruz) antibodies. Results were quantified on a Cyclone Phosphorimager (Perkin-Elmer).

2.8. Statistics

Data were expressed at the mean ± S.D., and analyzed by the Student's *t*-test. *P*-values below 0.05 were considered as statistically significant.

3. Results

3.1. GSTP1-1 expression during hemin-induced erythroid differentiation

Since we recently showed that the GSTP1-1 gene was differentially regulated during erythroid and megacaryocytic differentiation induced by anthracyclines [20], we investigated the effect of hemin, a prototypical inducer of erythroid differentiation. Hemoglobin synthesis increased in K562 cells in a time-dependent manner and after 4 days of treatment with hemin a 30 μM, 92.4% of the cells produced hemoglobin (Fig. 1). Differentiation was accompanied by marked growth inhibition (54.0%) and weak cellular cytotoxicity (3.5%) (Fig. 1). In parallel, quantification of northern and western blots (Fig. 2A–D) showed a significant increase of 70% ($P < 0.01$) and 20% ($P < 0.01$) for mRNA and protein expression, respectively, allowing to establish a link between GSTP1-1 expression and hemin-induced erythroid differentiation of K562 cells. As GSTP1-1 mRNA and protein levels spontaneously increase over 4 days under non-differentiating conditions (compare Fig. 1, lanes 1 and 2), we expressed GSTP1-1 mRNA and protein amounts relative to a control for each experimental time point in order to level for non-specific increases.

3.2. GATA-1 DNA binding activity and mRNA expression in hemin-induced differentiation of K562 cells

Previous results allowed us to explain differentiation-specific GSTP1-1 expression by the regulated binding of

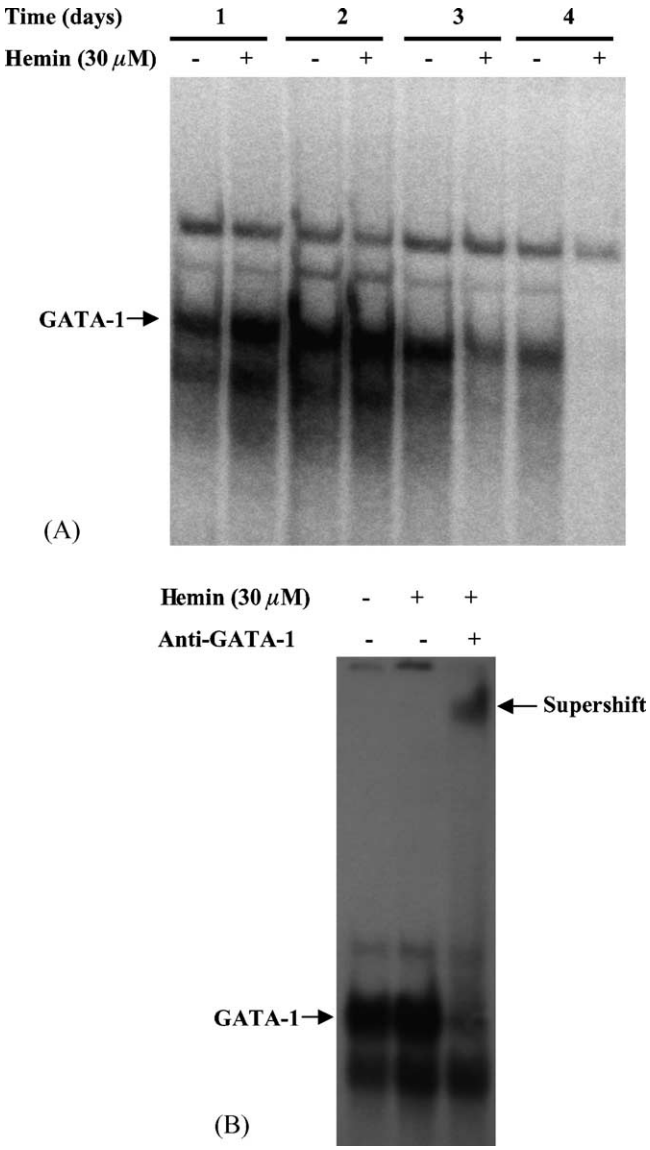

Fig. 3. Effect of hemin-induced erythroid differentiation on GATA-1 binding activity to GSTP1-1 promoter. (A) K562 cells were grown in medium alone or supplemented with 30 μM hemin for various times. Ten micrograms of nuclear extracts from control and treated K562 cells were incubated for 20 min at 4 °C with ^{32}P-labeled GATA probe. (B) Ten micrograms of nuclear extracts from treated or control K562 cells were incubated for 20 min at 4 °C with or without anti GATA-1 antibody and then for 20 min at 4 °C with ^{32}P-labeled GATA probe. The specific DNA–protein complex is indicated. Results shown are representative of three independent experiments.

GATA-1 to the promoter at a site located at −1208 relative to the transcriptional start site. In order to determine whether hemin regulates GSTP1-1 mRNA expression by the same molecular mechanism, we analyzed GSTP1-1 promoter/GATA-1 binding activity performing EMSA. Results showed a transient increase in GATA-1 binding activity after 24 h (Fig. 3A) followed by a sustained decrease after 72 h of treatment. Supershift experiments confirmed the presence of transcription factor GATA-1 in the hemin-induced binding complex (Fig. 3B). Results were confirmed by RT-PCR analysis showing a time-

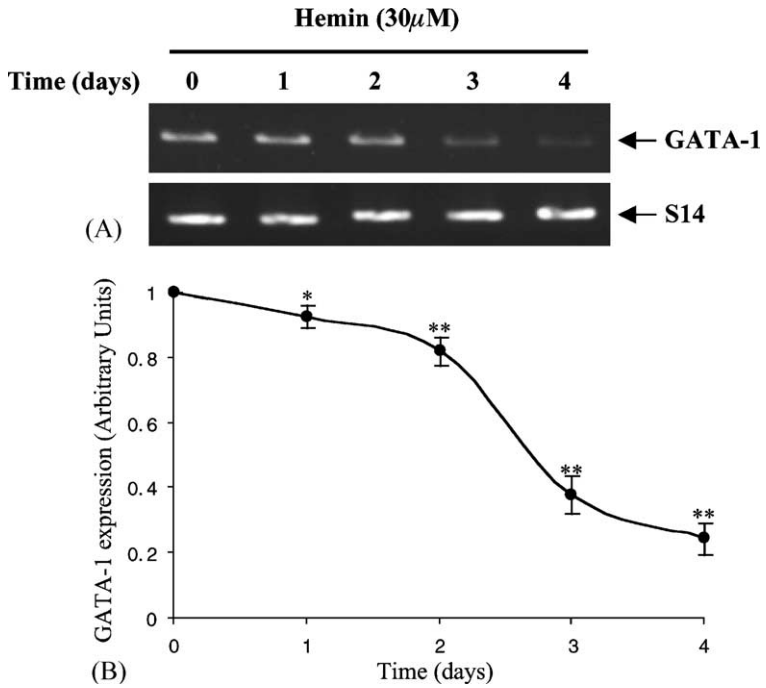

Fig. 4. Detection of GATA-1 gene expression by RT-PCR analysis. K562 cells were induced to differentiate towards the erythroid pathway with 30 μM hemin. (A) Total RNA was extracted and submitted to reverse transcription. RT products were used as templates for PCR amplification using gene specific sense and antisense primers for GATA-1. S14 amplification served as a control. Results shown are representative of three independent experiments. (B) PCR results were expressed as a ratio between GATA-1 and S14 amplification signals. Values are reported as the means (±S.D.) of three independent experiments. (*) and (**) indicate $P < 0.05$ and $P < 0.01$ compared to control, respectively.

dependent decrease (76%) of GATA-1 mRNA expression after 4 days of treatment (Fig. 4A).

3.3. NF-κB and AP-1 binding activity in hemin-treated K562 cells

Other transcription factors were shown to regulate GSTP1-1 gene transcription under various physiological conditions and could therefore be involved in GSTP1-1 mRNA over-expression during hemin-induced differentiation of K562 cells. We examined the effect of hemin treatment on NF-κB and AP-1 binding activities to the GSTP1-1 promoter. EMSA experiments showed that the interaction between GSTP1-1 promoter and transcription factors NF-κB (Fig. 5A) and AP-1 (Fig. 6A) was not affected during hemin treatment of K562 cells. TNFα-induced NF-κB binding, immunodepleted by anti-p50-NF-κB antibody, (Fig. 5B) and TPA-induced AP-1 binding supershifted by anti-cjun antibody (Fig. 6B) served as controls.

3.4. Effect of hemin on GSTP1-1 mRNA half-life

As the increase in GSTP1-1 gene expression could not be explained by enhanced binding of specific transcription factors in hemin-treated K562 cells, we hypothesized that the accumulation of GSTP1-1 mRNA could result from its stabilization. Relative half-life of GSTP1-1 mRNA was determined in hemin-treated and untreated K562 cells using the RNA polymerase II inhibitor actinomycin D. Decay of GSTP1-1 mRNA was analyzed by northern blot (Fig. 7A) and quantified. Results are represented as a linear regression to evaluate the relative half-life of GSTP1-1 mRNA which was 44.2 h in non-induced cells versus 92.6 h in hemin-treated K562 (Fig. 7B). Results allowed us to conclude that increased GSTP1-1 gene expression in hemin-induced erythroid differentiation of K562 cells was not due to transcriptional regulation mechanisms but to post-transcriptional stabilization of its mRNA.

4. Discussion

During the differentiation of erythroid cells, a maturation takes place leading to the elimination of organelles including the nucleus. In order to assure the regulated expression of vital proteins, mRNA stabilization is known to contribute to maintain physiological functions of the red blood cells. We previously showed that induction of erythroid differentiation by aclarubicin and doxorubicin leads to an increase in GSTP1-1 expression. In contrast, TPA, a megakaryocytic pathway inducer, inhibited constitutive GSTP1-1 expression at both mRNA and protein levels [20]. For this work, we used the canonical erythroid differentiating agent hemin.

The molecular effect of hemin at the transcriptional level was previously described by Partington and Patient using EMSA [30]. Indeed, GATA-1 DNA-binding activity to the

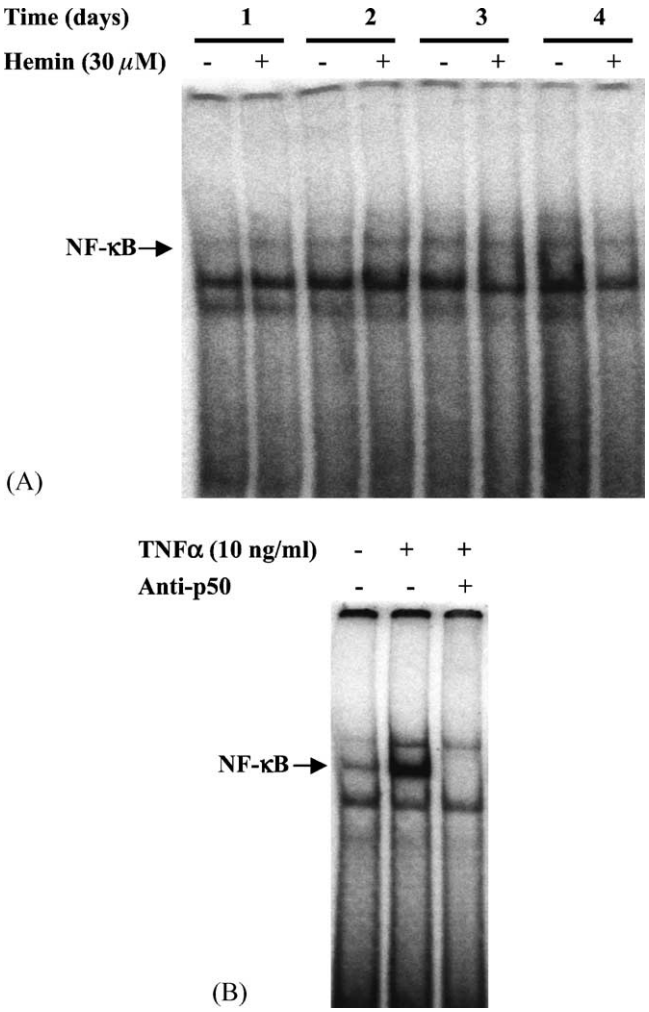

Fig. 5. Effect of hemin-induced erythroid differentiation on NF-κB binding activity. K562 cells were grown in medium alone or supplemented with 30 μM hemin for various times (A) or with TNFα at 10 ng/ml for 20 min as a positive control with or without an anti-p50 antibody (B). Ten micrograms of nuclear extracts from control and treated K562 cells were incubated for 30 min at 4 °C with ^{32}P-labeled probe. Results shown are representative of three independent experiments. Specific DNA-protein complexes are indicated.

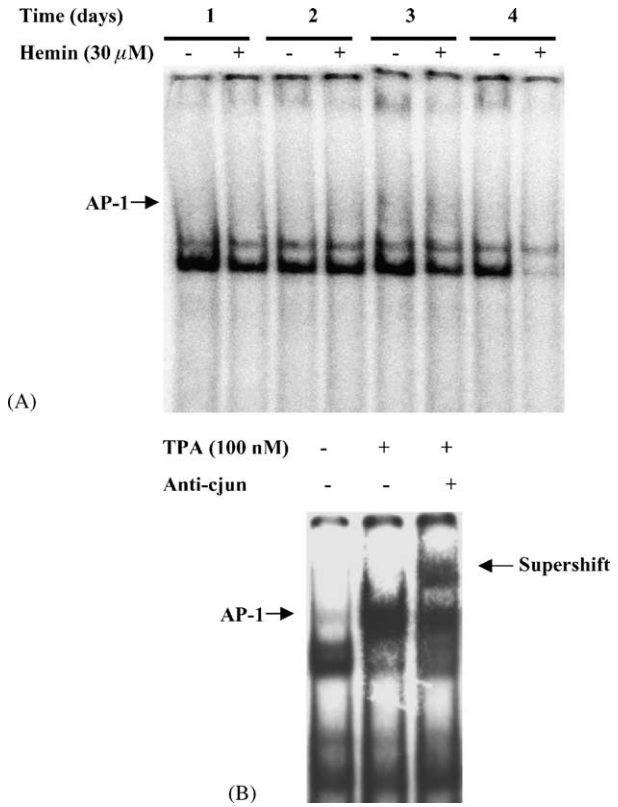

Fig. 6. Effect of hemin-induced erythroid differentiation on AP-1 binding activity. K562 cells were grown in medium alone or supplemented with 30 μM hemin for various times (A) or with TPA at 100 nM for 30 min as a positive control with or without an anti-cjun antibody (B). Ten micrograms of nuclear extracts from control and treated K562 cells were incubated for 30 min at 4 °C with ^{32}P-labeled probe. Results shown are representative of three independent experiments. Specific DNA–protein complexes are indicated.

α-globin gene promoter increased after induction of erythroid differentiation of K562 cells by 50 μM hemin. Our results confirm partially these findings as we observe an initial increase in GATA binding activity after 24 h of treatment. Our results obtained at a differentiating concentration of 30 μM show that a prolonged treatment by hemin induces a reduction of GATA-1 mRNA expression as well as transcription factor binding activity to the GSTP1-1 GATA site located at −1208 relative to the transcriptional start site. Site specific differences between both α-globin and GSTP1-1 GATA sites as well as differential phosphorylation of the GATA-1 factor could provide elements to explain a differential affinity of the GATA transcription factor for the GSTP1-1 gene promoter. Nevertheless, other post-transcriptional regulatory mechanisms should also be taken into consideration for a better understanding of the regulated expression of GSTP1-1.

Indeed GSTP1-1 mRNA appears to be already highly stable in K562 leukemia cells with a half-live of more then 40 h which is even further increasing to 92 h after hemin treatment and erythroid differentiation. Previous reports quantified GST mRNA half-lives which depend on the cellular model and the expressed isoform. Benbrahim-Tallaa et al. [31] reported that the mRNA half-life time was 18 h for GSTα in Sertoli cells and was unaffected by the presence of TNFα whereas FSH increased GSTα mRNA levels by increasing its mRNA stability to 119 h. According to Rogiers et al. [32] the half-life of rat GSTA1/A2 mRNA in control animals was 3.6 h, whereas it increased to 10.2 h in phenobarbital-treated animals. Schwartz and Norris [33] demonstrated that hGSTYBX, a μ class glutathione *S*-transferase gene, is expressed in the DDT1MF-2 hamster smooth muscle tumor cell line. Glucocorticoid induction stabilized the corresponding mRNA with a half-life of more than 48 h. Finally Jhaveri et al. reported higher GSTP1-1 mRNA stability in ER − HS578T cells compared to ER + MCF7 breast cancer cells [34]. Our results add GSTP1-1 mRNA to the erythroid-specific genes which are stabilized at the onset of differentiation. Stabilization of this mRNA during ery-

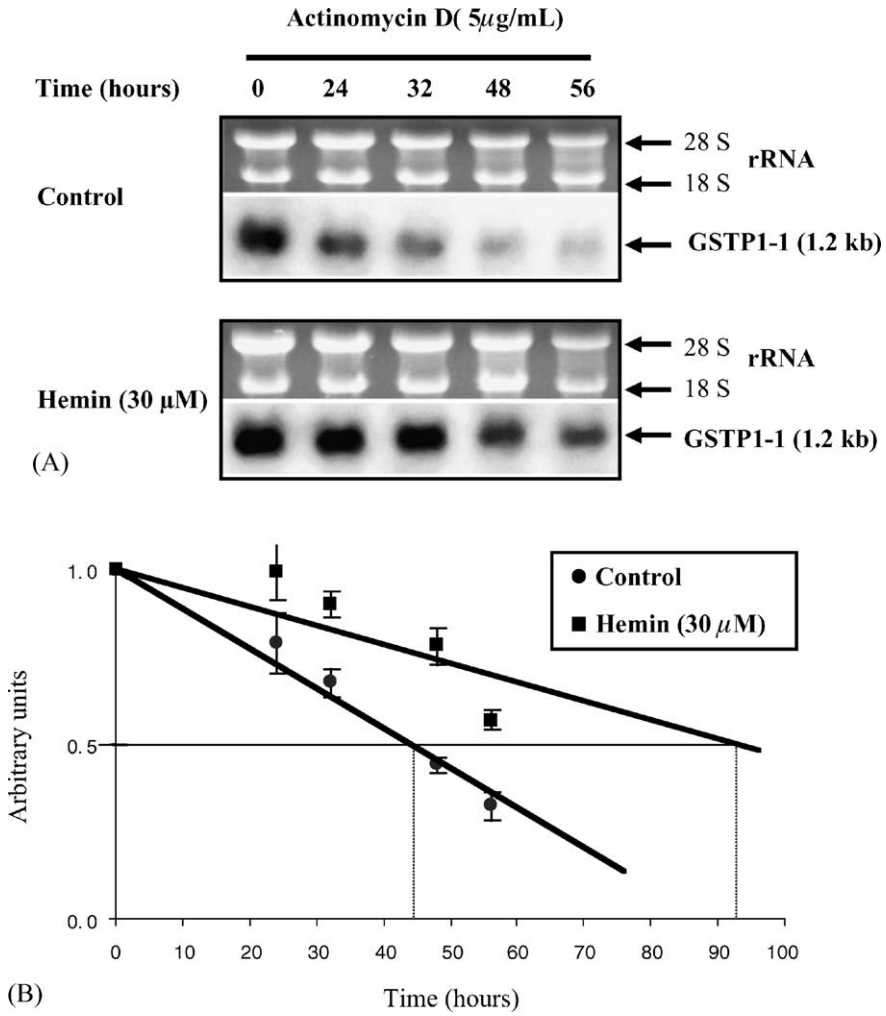

Fig. 7. Effect of hemin-induced erythroid differentiation of K562 cells on GSTP1-1 mRNA stability. Cells were grown in medium alone or in the presence of 30 μM hemin for 48 h. (A) Total RNA was isolated 24, 36, 48 and 56 h after actinomycin D addition (5 μg/ml) and analyzed by northern blot. Ethidium bromide-stained gel is shown as a loading control. (B) Northern blots were quantified by phosphorimaging and results were expressed as a linear regression of the means of three independent experiments in order to evaluate the relative half-life of GSTP1-1 mRNA. S.D. are indicated.

throid maturation raises curiosity about the potential detoxifying role of GSTP1-1 in mature erythrocytes which is the major GST isoform expressed by these cells [35].

Another example of mRNA stabilization by hemin was observed in the case of globin mRNA [36]. Moreover, during hemin-mediated erythroid differentiation of K562 cells, heat shock protein (HSP) 70 upregulation [37] was observed. Furthermore, Pirkkala et al. [38] demonstrated a multistep regulatory process of heat shock transcription factor (HSF) 2 gene expression. Indeed the increase in HSF2 protein levels is preceded by transcriptional induction of the HSF2 gene, accompanied by increased HSF2 mRNA stability after hemin-induced erythroid differentiation. This increase leads to an induced HSF-HSP interaction. Our results show that GSTP1-1 involved in oxidative stress response and detoxification against free radicals is also induced by hemin at both mRNA and protein levels and thus add GST to the enzymes involved in cellular protection during differentiation.

Other inducers of erythroid differentiation were previously described to regulate specific genes at the post-transcriptional level. Formerly obtained results reported that doxorubicin induced an increased stability of PBGD and GATA-1 mRNAs, whereas aclarubicin did not affect the half-lives of these mRNAs [39]. The same author provided evidence that GTP treatment led to a drastic increase of the γ-globin mRNA half-life. This stabilizing effect of GTP was mediated via the 3′-untranslated region (UTR) of the γ-globin mRNA. In this cellular model, an early activation of γ-globin gene transcription was followed by a stabilization of its mRNA [40].

Molecular determinants of increased mRNA stability were previously described for chénaisselected erythroid specific genes including the human globin mRNA [36,41], 15-lipoxygenase (LOX) [42] as well as the 5-aminolevulinate synthetase (eALAS) mRNA which was shown to be sufficient to confer translational control to a reporter mRNA both in transfected MEL cells and in vitro [43].

γ-Glutamyltransferase, another enzyme of the glutathione pathway was previously described to be regulated at the post-transcriptional level [44,45]. The 3′ UTR of GSTP1-1 encompasses 76 nucleotides including a 13 nt long pyrimidine-rich region. A similar region is involved in the regulation of α-globin mRNA stabilization by (PolyC) binding protein αCP [46]. Whether this type of sequence regulates the increased stability of the GSTP1-1 mRNA remains to be elucidated.

Chenais et al. [47] recently suggested that oxidative stress, generated by differentiating agents including aclarubicin, doxorubicin and butyric acid, is involved in the first step of differentiation of K562 cells towards the erythroid lineage. Hemin was also previously shown to be inducing oxidative stress in rat liver [48] which could explain the induction of the GSTP1-1 gene at the onset of the differentiation process.

Altogether, GSTP1-1 expression appears to be regulated by the combined influence of both transcriptional and post-transcriptional mechanisms during hemin-induced erythroid differentiation in K562 cells. Stabilization of mRNA of genes involved in glutathione-related anti-cancer drug resistance raises questions about the development of differentiated but chemoresistant cells potentially lowering the success of promising differentiation therapies. In order to address this important issue, which to our knowledge is only poorly understood at the present time, future studies are needed to correlate induced GSTPl-1-overexpression mechanisms to differentiation and chemoresistance.

Acknowledgments

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References

[1] Hayes JD, Pulford DJ. The glutathione *S*-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol Biol 1995;30(6):445–600.

[2] Sheehan D, Meade G, Foley VM, Dowd CA. Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem J 2001;360(Pt 1):1–16.

[3] Whalen R, Boyer TD. Human glutathione *S*-transferases. Semin Liver Dis 1998;18(4):345–58.

[4] Henderson CJ, Smith AG, Ure J, Brown K, Bacon EJ, Wolf CR. Increased skin tumorigenesis in mice lacking pi class glutathione *S*-transferases. Proc Natl Acad Sci USA 1998;95(9):5275–80.

[5] Sato K. Glutathione transferases as markers of preneoplasia and neoplasia. Adv Cancer Res 1989;52:205–55.

[6] Sauerbrey A, Volm M, Zintl F. Expression and clinical significance of resistance proteins in initial acute lymphatic leukemia (ALL) in childhood. Klin Padiatr 1995;207(4):230–4.

[7] Monden N, Abe S, Sutoh I, Hishikawa Y, Kinugasa S, Nagasue N. Prognostic significance of the expressions of metallothionein, glutathione-*S*-transferase-pi, and P-glycoprotein in curatively resected gastric cancer. Oncology 1997;54(5):391–9.

[8] Ranganathan S, Tew KD. Immunohistochemical localization of glutathione *S*-transferases alpha, mu, and pi in normal tissue and carcinomas from human colon. Carcinogenesis 1991;12(12):2383–7.

[9] Schisselbauer JC, Silber R, Papadopoulos E, Abrams K, LaCreta FP, Tew KD. Characterization of glutathione *S*-transferase expression in lymphocytes from chronic lymphocytic leukemia patients. Cancer Res 1990;50(12):3562–8.

[10] Ali-Osman F, Brunner JM, Kutluk TM, Hess K. Prognostic significance of glutathione *S*-transferase pi expression and subcellular localization in human gliomas. Clin Cancer Res 1997;3(12 Pt 1):2253–61.

[11] Clapper ML, Szarka CE. Glutathione *S*-transferases—biomarkers of cancer risk and chemopreventive response. Chem Biol Interact 1998;111–112:377–88.

[12] Tew KD. Glutathione-associated enzymes in anticancer drug resistance. Cancer Res 1994;54(16):4313–20.

[13] Puchalski RB, Fahl WE. Expression of recombinant glutathione *S*-transferase pi, Ya, or Ybl confers resistance to alkylating agents. Proc Natl Acad Sci USA 1990;87(7):2443–7.

[14] Moscow JA, Townsend AJ, Cowan KH. Elevation of pi class glutathione *S*-transferase activity in human breast cancer cells by transfection of the GST pi gene and its effect on sensitivity to toxins. Mol Pharmacol 1989;36(1):22–8.

[15] Adler V, Yin Z, Fuchs SY, Benezra M, Rosario L, Tew KD, et al. Regulation of JNK signaling by GSTp. EMBO J 1999;18(5):1321–34.

[16] Morrow CS, Goldsmith ME, Cowan KH. Regulation of human glutathione *S*-transferase pi gene transcription: influence of 5′-flanking sequences and trans-activating factors which recognize AP-1-binding sites. Gene 1990;88(2):215–25.

[17] Moffat GJ, McLaren AW, Wolf CR. Spl-mediated transcriptional activation of the human Pi class glutathione *S*-transferase promoter. J Biol Chem 1996;271(2):1054–60.

[18] Borde-Chiche P, Diederich M, Morceau F, Wellman M, Dicato M. Phorbol ester responsiveness of the glutathione *S*-transferase PI gene promoter involves an inducible c-jun binding in human K562 leukemia cells. Leuk Res 2001;25(3):241–7.

[19] Morceau F, Duvoix A, Delhalle S, Schnekenburger M, Dicato M, Diederich M. Regulations of glutathione *S*-transferase Pl-1 gene expression by NF-kappaB in tumor necrosis factor alpha-treated K562 leukemia cells. Biochem Pharmacol 2004;67(7):1227–38.

[20] Schnekenburger M, Morceau F, Duvoix A, Delhalle S, Trentesaux C, Dicato M, et al. Expression of glutathione *S*-transferase Pl-1 in differentiating K562: role of GATA-1. Biochem Biophys Res Commun 2003;311(4):815–21.

[21] Lozzio BB, Lozzio CB, Bamberger EG, Feliu AS. A multipotential leukemia cell line (K-562) of human origin. Proc Soc Exp Biol Med 1981;166(4):546–50.

[22] Paquette RL, Koeffler HP. Differentiation therapy. Hematol Oncol Clin North Am 1992;6(3):687–706.

[23] Beere HM, Hickman JA. Differentiation: a suitable strategy for cancer chemotherapy? Anticancer Drug Des 1993;8(4):299–322.

[24] Degos L. Differentiating agents in the treatment of leukemia. Leuk Res 1990;14(8):717–9.

[25] Leszczyniecka M, Roberts T, Dent P, Grant S, Fisher PB. Differentiation therapy of human cancer: basic science and clinical applications. Pharmacol Ther 2001;90(2–3):105–56.

[26] Fibach E, Kollia P, Schechter AN, Noguchi CT, Rodgers GP. Hemin-induced acceleration of hemoglobin production in immature cultured erythroid cells: preferential enhancement of fetal hemoglobin. Blood 1995;85(10):2967–74.

[27] Mustajoki P, Nordmann Y. Early administration of heme arginate for acute porphyric attacks. Arch Intern Med 1993;153(17):2004–8.
[28] Orkin SH, Swan D, Leder P. Differential expression of alpha- and beta-globin genes during differentiation of cultured erythroleukemic cells. J Biol Chem 1975;250(22):8753–60.
[29] Muller MM, Schreiber E, Schaffner W, Matthias P. Rapid test for in vivo stability and DNA binding of mutated octamer binding proteins with 'mini-extracts' prepared from transfected cells. Nucleic Acids Res 1989;17(15):6420.
[30] Partington GA, Patient RK. Phosphorylation of GATA-1 increases its DNA-binding affinity and is correlated with induction of human K562 erythroleukaemia cells. Nucleic Acids Res 1999;27(4):1168–75.
[31] Benbrahim-Tallaa L, Tabone E, Tosser-Klopp G, Hatey F, Benahmed M. Glutathione *S*-transferase alpha expressed in porcine Sertoli cells is under the control of follicle-stimulating hormone and testosterone. Biol Reprod 2002;66(6):1734–42.
[32] Rogiers V, Akrawi M, Vercruysse A, Phillips IR, Shephard EA. Effects of the anticonvulsant, valproate, on the expression of components of the cytochrome-P-450-mediated monooxygenase system and glutathione *S*-transferases. Eur J Biochem 1995;231(2):337–43.
[33] Schwartz DA, Norris JS. Glucocorticoid, androgen, and retinoic acid regulation of glutathione *S*-transferase gene expression in hamster smooth muscle tumor cells. Biochem Biophys Res Commun 1992; 184(2):1108–13.
[34] Jhaveri MS, Stephens TE, Morrow CS. Role of posttranscriptional processes in the regulation of glutathione *S*-transferase PI gene expression in human breast cancer cells. Biochem Biophys Res Commun 1997;237(3):729–34.
[35] Guthenberg C, Mannervik B. Glutathione *S*-transferase (transferase pi) from human placenta is identical or closely related to glutathione *S*-transferase (transferase rho) from erythrocytes. Biochim Biophys Acta 1981;661(2):255–60.
[36] Bonanou-Tzedaki SA, Sohi MK, Arnstein HR. The effect of haemin on RNA synthesis and stability in differentiating rabbit erythroblasts. Eur J Biochem 1984;144(3):589–96.
[37] Theodorakis NG, Zand DJ, Kotzbauer PT, Williams GT, Morimoto RI. Hemin-induced transcriptional activation of the HSP70 gene during erythroid maturation in K562 cells is due to a heat shock factor-mediated stress response. Mol Cell Biol 1989;9(8):3166–73.
[38] Pirkkala L, Alastalo TP, Nykanen P, Seppa L, Sistonen L. Differentiation lineage-specific expression of human heat shock transcription factor 2. FASEB J 1999;13(9):1089–98.
[39] Morceau F, Chenais B, Gillet R, Jardillier JC, Jeannesson P, Trentesaux C. Transcriptional and posttranscriptional regulation of erythroid gene expression in anthracycline-induced differentiation of human erythroleukemic cells. Cell Growth Differ 1996;7(8):1023–9.
[40] Morceau F, Dupont C, Palissot V, Borde-Chiche P, Trentesaux C, Dicato M, et al. GTP-mediated differentiation of the human K562 cell line: transient overexpression of GATA-1 and stabilization of the gamma-globin mRNA. Leukemia 2000;14(9):1589–97.
[41] Yu J, Russell JE. Structural and functional analysis of an mRNP complex that mediates the high stability of human beta-globin mRNA. Mol Cell Biol 2001;21(17):5879–88.
[42] Ostareck-Lederer A, Ostareck DH, Standart N, Thiele BJ. Translation of 15-lipoxygenase mRNA is inhibited by a protein that binds to a repeated sequence in the 3' untranslated region. EMBO J 1994; 13(6):1476–81.
[43] Melefors O, Goossen B, Johansson HE, Stripecke R, Gray NK, Hentze MW. Translational control of 5-aminolevulinate synthase mRNA by iron-responsive elements in erythroid cells. J Biol Chem 1993; 268(8):5974–8.
[44] Chobert MN, Grondin G, Brouillet A, Laperche Y, Beaudoin AR. Control of gamma-glutamyl transpeptidase expression by glucocorticoids in the rat pancreas. Correlation with granule formation. J Biol Chem 1996;271(21):12431–7.
[45] Diederich M, Wellman M, Visvikis A, Puga A, Siest G. The 5′ untranslated region of the human gamma-glutamyl transferase mRNA contains a tissue-specific active translational enhancer. FEBS Lett 1993;332(1–2):88–92.
[46] Kong J, Ji X, Liebhaber SA. The KH-domain protein alpha CP has a direct role in mRNA stabilization independent of its cognate binding site. Mol Cell Biol 2003;23(4):1125–34.
[47] Chenais B, Andriollo M, Guiraud P, Belhoussine R, Jeannesson P. Oxidative stress involvement in chemically induced differentiation of K562 cells. Free Radic Biol Med 2000;28(1):18–27.
[48] Suliman HB, Carraway MS, Velsor LW, Day BJ, Ghio AJ, Piantadosi CA. Rapid mtDNA deletion by oxidants in rat liver mitochondria after hemin exposure. Free Radic Biol Med 2002;32(3):246–56.