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Relationship between the induction of leukemia cell differentiation and the enhancement of reporter gene expression in 3T3 Swiss cells

Kimiko Ishiguro and Alan C. Sartorelli*

Department of Pharmacology and Developmental Therapeutics Program, Cancer Center, Yale University School of Medicine, New Haven, CT 06520, USA

Abstract

The histone deacetylase (HDAC) inhibitor trichostatin A (TSA) and hexamethylene bisacetamide (HMBA), which lacks HDAC inhibitory activity, both possess the capacity to induce leukemia cell differentiation and to enhance the expression of a wide range of transiently transfected reporter genes in 3T3 Swiss cells. In addition, known inducers of leukemia cell differentiation, including hypoxanthine, diazepam, 6-thioguanine and phorbol 12-myristate 13-acetate, also exhibited the ability to enhance reporter gene expression, while randomly chosen compounds that did not induce leukemia cell differentiation did not enhance reporter gene expression. The activity of TSA in the transfection system was modified by co-expression of histone acetyltransferase p300 and HDAC1; whereas, that of HMBA was enhanced by co-expression of the TATA-binding protein TBP. The stimulatory effects of diverse chemical inducers on transiently transfected genes suggest the existence of multiple exploitable targets for the selection of novel inducers of differentiation that function as modulators of gene activity.

Keywords

Induction of differentiation; Hexamethylene bisacetamide; Diazepam; 6-Thioguanine; Trichostatin A; Phorbol 12-myristate 13-acetate; Reporter gene expression

1. Introduction

Neoplastic tumors consist of populations of malignant cells with proliferative capabilities whose movement along the maturation pathway is impeded by alterations in regulatory mechanisms. The demonstration that certain chemical agents exhibit the ability to induce the terminal differentiation of a variety of transformed cell lines indicates that the differentiation block imposed in tumor cells by the carcinogenic process is not necessarily irreversible [1]. Thus, cancer therapy based upon the premise that the conversion of malignant cells to more mature end-stage forms lessens their proliferative and invasive capacity provides an attractive alternative to conventional cytotoxic cancer treatment (see, for example [2]). This approach termed “differentiation therapy” has culminated in the successful use of the vitamin A derivative all-*trans* retinoic acid (ATRA) as a primary treatment for patients with acute promyelocytic leukemia (APL) [3,4]. Although differentiation therapy has received much attention due in part to the remarkable efficacy of ATRA in APL, further progress in expanding the applicability of this approach has been hampered by an inability to identify potential

*Corresponding author. Tel.: +1 203 785 4533; fax: +1 203 737 2045. alan.sartorelli@yale.edu (A.C. Sartorelli).

therapeutic targets due to a lack of understanding of (a) the decision mechanism by which stem cells either undergo self-renewal or commit to a differentiation pathway, (b) the mechanisms by which tumors are arrested at immature stages of development, and (c) the mechanisms by which some chemical agents overcome a cellular impediment to complete a differentiation program, thereby inducing the maturation of various types of tumor cells.

The inhibitors of HDACs, including sodium butyrate and TSA, are inducers of growth arrest, differentiation, and/or apoptosis in a variety of transformed cell lines [5] and some fatty acids and synthetic hydroxamates are currently in clinical trial as potential cancer therapeutics [6]. In contrast to the HDAC inhibitors with a definitive mode of action, prototypical inducers of differentiation such as dimethylsulfoxide (DMSO) and HMBA are devoid of the ability to inhibit HDACs and yet are characterized by their ability to induce differentiation and/or apoptosis [7,8]. The mode of action of these prototypical inducers has escaped a great deal of understanding, not due to a lack of interest, but due to the lack of an experimental assay system reflective of their site(s) of action. We have discovered that HMBA and DMSO exhibit the capacity to enhance the expression of a wide range of transiently transfected genes in 3T3 Swiss cells in a manner analogous to that of TSA, and that HMBA and DMSO enhance transcriptional initiation of reporter genes using nuclear run-on assays [8]. These findings suggest that the primary site of action of the prototypical inducers involves a direct interaction with chromatin.

In this report, a relatively large number of chemical agents were employed to demonstrate the existence of a relationship between the ability to induce leukemia cell differentiation and the capacity to enhance the expression of a wide range of reporter genes in 3T3 Swiss cells. The co-transfection of various transcription-related factors in a transient transfection system, and gel shift assays were employed to gain information on the mechanisms of their stimulatory actions on reporter genes. These studies demonstrated the convergence of different structural types of chemical inducers of differentiation as modulators of gene activity.

2. Materials and methods

2.1. Cell lines and differentiation assay

Friend murine erythroleukemia (F-MEL) 745-PC-4 cells [9] and HGPRT deficient 745-TG-11 cells [10] were the gift of David Housman (MIT, Cambridge, MA). Cells were maintained in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum at 37 °C in a humidified atmosphere containing 10% CO₂. For induction of differentiation, cells at a density of $7 \times 10^4 \text{ ml}^{-1}$ were exposed to chemical inducers for 3 days. Cell numbers were determined using a Coulter Counter with a Multisizer II analyzer (Beckman, Hialeah, FL). Hemoglobin production was assessed by benzidine staining as described previously [11]. HMBA (Sigma, St. Louis, MO) was dissolved in water at a concentration of 500 mM. TSA (Wako Chemicals, Richmond, VA) was dissolved in ethanol at a concentration of 2 mg/ml and added to cells, with a constant final concentration of 0.1% ethanol. Hyp was solubilized in 0.75N NaOH at a concentration of 600 mM. TGua was prepared in 0.1N NaOH at a concentration of 75 mM. PMA (Sigma) was dissolved in DMSO at a level of 1 mg/ml and added to cells, with a constant final concentration of 0.1% DMSO. Diazepam (Research Biochemicals International, Natick, MA) was dissolved in ethanol at a level of 100 mM. Growth inhibition caused by Hyp and diazepam at the levels of 7.5 and 0.2 mM, respectively, was in part due to the solvents.

2.2. Transient transfection of reporter genes in 3T3 Swiss cells

The method to measure enhancement of luciferase activity of transiently transfected reporter genes by chemical agents in 3T3 Swiss cells has been described in detail [8]. Reporter plasmids

pGL3 control, pRL-TK, and pRL-CMV were obtained from Promega (Madison, WI). Reporter plasmids pNF- κ B-Luc and p53-Luc were from Stratagene (La Jolla, CA). Construction of globin gene reporter plasmid HS2-421 β^m has been documented [8]. For co-transfection of transcriptional modulators, including p300, HDAC1, TBP, and TFIIB, 0.2 μ g of each reporter plasmid HS2-421 β^m and expression plasmid containing each cDNA under the control of the CMV promoter, were co-transfected. Human *HDAC1* cDNA in pcDNA3 was the kind gift of Donald E. Ayer (University of Utah, Salt Lake City, UT). Human wild-type *p300* cDNA in pCMV β was from Upstate Biotechnology (Lake Placid, NY). Murine *TBP* (BC012685) and *TFIIB* (BC016637) cDNAs in pCMV-SPORT6 were purchased from Open Biosystems (Huntsville, AL).

Standard errors of triplicate samples of luciferase assays in the transient transfection system were typically less than 10%. Each experiment was repeated at least twice for reproducibility and a representative experiment has been shown.

2.3. Gel shift assay

3T3 Swiss cells were seeded at 2×10^6 per 75 cm² flask, cultured overnight and treated with various chemical agents for 4 h. Cells were trypsinized and nuclear extracts (50 μ l) were prepared as described previously [12]. AP-1 and NF- κ B consensus oligonucleotides (Promega) were 3'-end labeled with digoxigenin (DIG)-ddUTP using a DIG gel shift kit (Roche Applied Science, Indianapolis, IN). Two micro-liters of nuclear extract were mixed with 0.0308 pmol of DIG-labeled oligonucleotides, and reaction products were separated on Novex 6% acrylamide DNA retardation gels (Invitrogen, Carlsbad, CA), transferred to a nylon membrane and visualized using anti-DIG antibody and CDP-Star (Roche), according to the manufacturers' instructions.

2.4. Preparation of histones and western analysis

F-MEL cells at a density of 4×10^5 ml⁻¹ in a volume of 20 ml were treated with chemical agents for 8 h. Core histones were extracted with 0.2 M H₂SO₄ from nuclear pellets, subjected to 15% polyamide gel electrophoresis and analyzed by Western blotting as reported previously in detail [8].

2.5. Northern hybridization

Total cellular RNA was extracted from 6 to 8×10^6 cells using Trizol reagent (Invitrogen), separated by agarose/formaldehyde gel electrophoresis, stained with acridine orange, transferred to a nylon membrane, and hybridized with DIG-labeled antisense RNA probes as indicated [8]. The 579 bp-c-myc probe corresponding to exon 3 of the murine *c-myc* cDNA (BC002043, Open Biosystems, Huntsville, AL) was inserted into pCRII-TOPO (Invitrogen) by TA cloning. The 599 bp-coding region of the histone H1⁰ probe was generated from DMSO treated F-MEL RNA using the Titan One-Tube RT-PCR system (Roche), followed by TA cloning of the fragment into pCRII-TOPO. All fragments generated by PCR were verified by DNA sequencing.

3. Results

In addition to the prototypical inducers of differentiation DMSO and HMBA, several other chemical agents have been identified as initiators of the maturation of F-MEL cells. These include the purine hypoxanthine (Hyp), the purine antimetabolite 6-thioguanine (TGua) [10], and the tranquilizer diazepam [13]. The differentiation inducing properties of these chemicals in F-MEL cells are summarized in Table 1. HMBA was the most effective inducer of differentiation, producing over 95% benzidine positive cells at concentrations over 5 mM. Hyp was similar to HMBA in potency but less effective than HMBA, causing 35% benzidine

positive cells at 7.5 mM. Although TGua was growth-inhibitory to wild-type F-MEL cells (IC_{50} , 2 μ M), TGua was effective in initiating the maturation of hypoxanthine-guanine phosphoribosyltransferase (HGPRT) deficient F-MEL cells. TGua and diazepam (their effective concentrations, 0.1–0.2 mM) were ~30 times more potent than HMBA. DMSO was the least potent, requiring concentrations of 1.5–2.5% (211–352 mM) to induce differentiation.

The inhibitors of HDAC have been reported to stimulate the expression of a variety of reporter genes in transient transfection systems [14–16]. In an analogous manner, DMSO and HMBA enhance the expression of reporter genes irrespective of the promoter type in 3T3 Swiss cells [8]. To ascertain whether this phenomenon was produced by other chemical inducers, 3T3 Swiss cells were transfected with a reporter construct pGL3 control or pNF- κ B, and treated with various inducers for 20 h. As shown in Fig. 1, the prototypical inducers of differentiation DMSO and HMBA, as well as Hyp and diazepam all increased the expression of the reporter genes at a concentration range comparable to that required for the induction of differentiation (Table 1). We attempted to measure the activity of TGua in HGPRT[−] 3T3 Swiss cells, a system deemed comparable to HGPRT[−] F-MEL cells. However, we were unable to successfully develop TGua resistant clones from 3T3 Swiss cells. Therefore, TGua was tested in wild-type 3T3 Swiss cells in which it enhanced the expression of the pGL3 control and pNF- κ B by 3.9- and 2.2-fold, respectively, at a concentration of 0.05 mM. Since reporter gene assays require a relatively short (20 h) incubation period with chemical agents, we believe that the cellular capacity to process and express transfected DNA in TGua-treated cells is still intact, and that the fold increase values are meaningful. Therefore, all of the inducers of differentiation listed in Table 1 possessed the capacity to enhance the expression of transiently transfected genes in 3T3 Swiss cells.

To ensure that a relationship existed between the capacity of chemical agents to induce the differentiation of F-MEL cells and their ability to enhance reporter gene expression in 3T3 Swiss cells, a spectrum of chemical agents were randomly chosen and subjected to both differentiation assay in F-MEL cells and reporter gene assay using pGLC control and pNF- κ B in 3T3 Swiss cells over a range of non-toxic to toxic concentrations. The agents which were inactive in the differentiation assay, including dimethylformamide, spermine, spermidine, okadaic acid, staurosporin, rapamycin, cyclosporin A, tumor necrosis factor- α , lipopolysaccharide from *Escherichia coli* 0111, forskolin, and PD 98059, were also inactive in the reporter gene assay.

During the course of experiments described above, the canonical protein kinase C (PKC) activator, phorbol 12-myristate 13-acetate (PMA) was found to simulate the expression of the pGLC control and pNF- κ B. Although PMA is not an inducer of the differentiation of F-MEL cells [17], this agent is a potent inducer of differentiation of several myeloid leukemia cell lines [18]. Like TSA and HMBA, PMA possessed the capacity to enhance a wide range of reporter gene expressions (Fig. 2A). The types of promoters in the reporter genes used in this study are summarized in Table 2 for clarity. Since the modes of action of HMBA, TSA and PMA are distinctive, it was of interest to examine how these agents affected the expression of transfected genes. To this end, the kinetics of stimulation of reporter gene expression by these agents was measured. As shown in Fig. 2B, despite the difference in their target sites of action, the three agents exhibited similar kinetics; thus, stimulation was gradual, reached a maximum at 18 h and declined at 24 h.

To ascertain whether the action of PMA was mediated through the activation of PKC, cells were exposed to PMA, HMBA, and TSA in the simultaneous presence of the selective inhibitor of PKC, bisindolylmaleimide II (BII) [19]. The activity of PMA was reduced by BII in a concentration-dependent manner; whereas, BII slightly enhanced reporter gene expression in

the absence as well as the presence of TSA and HMBA (Fig. 2C). These results imply that the stimulatory action of PMA on the reporter genes requires PKC activation.

One of the possible mechanisms by which these agents enhance reporter gene expression is through an increase in the abundance or the activity of the recognition factor(s) for the promoter sequence necessary for transcription. A gel shift assay using the NF- κ B consensus sequence was carried out to evaluate this possibility. Since PMA is known to induce the activity of the AP-1 transcription factor [20], the AP-1 consensus sequence was used as a positive control. Under conditions in which PMA treatment specifically produced an increase in the binding activity of the AP-1 consensus sequence (Fig. 2D right), a slight increase in the binding activity for the NF- κ B consensus sequence was consistently observed in the nuclear extracts from PMA and TSA treated cells (Fig. 2D left).

TSA causes hyperacetylation of lysine residues in the N-terminal tails of core histones in cultured cells as a result of inhibition of the activities of class I, II and IV HDACs [6]. To confirm that the enhancers of reporter gene expression other than TSA do not mediate their effects through hyperacetylation of histones, the acetylation status of histones in F-MEL cells exposed to various enhancers was determined. As shown in Fig. 3A, TSA caused hyperacetylation of histone H3 (Lys 9) and H4 (Lys 5), and reciprocal hypophosphorylation of histone H3 (Ser 10), while DMSO, HMBA, diazepam, Hyp, TGua and PMA did not alter the co-valent modifications of these histones.

To gain information on whether the target site of HMBA interacts with that of TSA, the combination of HMBA and TSA was evaluated using the transient transfection system. Fig. 3B left panel shows that, although the concentration response curve of HMBA in the range of 0–15 mM as a single agent was sigmoidal (open circles), the addition of a fixed concentration of TSA (15 nM, open squares) resulted in a concentration response curve that was nearly linear. Fig. 3B right panel shows that, against graded concentrations of TSA, whose concentration response curve was linear from 0 to 15 nM (open circles), addition of a fixed concentration of HMBA (7.5 mM, open squares) produced essentially additive effects. These combination studies indicate that the target site(s) of HMBA is saturable and that the actions of TSA and HMBA are additive to synergistic.

Although the actions of both TSA and HMBA on transfected genes are mediated at the transcriptional level [8], the *trans*-acting factors involved in their activities are undefined. To this end, expression plasmids encoding several general transcription factors [21] and co-activators/co-repressors [22] were co-transfected with the reporter construct HS2-421 β^m , and the effects of the co-expressed transcription related factors on the activities of TSA, HMBA and PMA were evaluated. Co-transfection of the transcriptional co-activator HAT p300 [23] and co-repressor HDAC1 [24], positively and negatively, respectively, modulated the activity of TSA, while these two factors produced similar effects in untreated, HMBA- and PMA-treated cells (Fig. 3C left). In contrast, the co-transfection of the TATA-binding protein TBP [25] up-regulated the activity of HMBA to the largest extent (Fig. 3C right). Co-transfection of TFIIB, that regulates transcription by self-acetylation [26], gave a slight enhancement of the activity of TSA, while co-transfection of TFIIB reduced reporter gene expression in untreated, HMBA- and PMA-treated cells (Fig. 3C right).

In contrast to the effects of chemical inducers on the expression of transfected genes that are exclusively stimulatory, the prototypical inducers DMSO and HMBA are known to up- as well as down-regulate certain sets of endogenous genes [7]. One of the up-regulated genes is *histone H1⁰* [27], while one of the down-regulated genes is the proto-oncogene *c-myc* [28]. Fig. 4A left panel shows the levels of histone H1⁰ and *c-myc* mRNAs in F-MEL cells treated with the various inducers for 8 h. PMA, which was not an inducer in F-MEL cells, did not alter the

levels of these mRNAs. HMBA and TSA caused the most pronounced increase in the histone H1⁰ mRNA level, while HMBA and DMSO caused the most pronounced decrease in the c-myc mRNA level. The time course of histone H1⁰ mRNA accumulation by TSA and by HMBA (Fig. 4A right panel) coincided with the time course of histone hyperacetylation caused by TSA (Fig. 4B). In contrast, the reduction in c-myc mRNA level produced by HMBA and by TSA occurred within 1.5 h, timing inconsistent with that of histone hyperacetylation produced by TSA.

4. Discussion

In this report, we have demonstrated that at least three distinctive groups of differentiation inducers exhibit the capacity to enhance transiently transfected genes in 3T3 Swiss cells irrespective of the promoter type. These include inhibitors of HDACs, well-known inducers of differentiation currently in clinical trial as antitumor agents [6]; the prototypical inducers of differentiation DMSO and HMBA devoid of HDAC inhibitory activity; and the PKC activator PMA. We believe that Hyp, diazepam and TGua, belong to the second group with DMSO and HMBA, because they are distinguished from TSA and PMA, in their inability to cause hyperacetylation of core histones (Fig. 3A) and in their ability to cause differentiation of F-MEL cells, respectively (Table 1). Although PMA does not induce the differentiation of F-MEL cells, this agent is a potent inducer of differentiation of several myeloid cell lines [18]. Thus, a relationship appears to exist between the capacity to induce cellular differentiation and to enhance transiently transfected reporter genes in 3T3 Swiss cells.

Inhibitors of HDAC cause hyperacetylation of the N-terminal tails of core histones. The discovery that genetically determined transcriptional co-activators and co-repressors express intrinsic HAT and HDAC activity, respectively, has implicated acetylation events as a major component of transcriptional regulation [22]. Since clear evidence exists that transcriptional activation requires acetylation of the N-terminal tail domain of core histones, particularly those of histones H3 and H4 in a reconstituted transcription system [29], it is reasonable that HDAC inhibitors such as TSA and sodium butyrate possess the capacity to stimulate transcription of a wide variety of transfected genes. Accordingly, the activation of a reporter gene by TSA was either enhanced or suppressed by co-transfection of the p300 HAT or HDAC1 expression plasmid, respectively (Fig. 3C). In contrast to HDAC inhibitors, the prototypical inducers of differentiation such as DMSO and HMBA are not implicated in covalent modifications of histones and yet possess the ability to non-specifically enhance reporter gene expression. Using nuclear run-on assays, we have provided evidence that these agents act at the level of transcriptional initiation [8]. Compatible with these observations, the activity of HMBA in transient transfections was greatly enhanced by co-transfection of the TATA-binding protein TBP (Fig. 3C). Since the reporter construct (HS2-421β^m) used in the experiment contains a TATA-box, a *cis*-acting component identified to be crucial for the transient expression in HeLa cells [30], it is plausible that HMBA enhances reporter gene expression by facilitating the formation of an initiation complex for transcription. We speculate that the primary action of the prototypical inducers HMBA and DMSO, as well as diazepam, Hyp and TGua, is to non-covalently interact with chromatin to produce a milieu that leads to chromatin destabilization, which stimulates transcription in a manner analogous to that caused by hyperacetylation of core histones.

In contrast to acetylation events, the precise mechanism by which the phosphorylation event produced by PMA, the activator of PKC, stimulates reporter gene expression is less clear. PMA was analogous to TSA and HMBA in its capacity to enhance reporter gene expression in a non-specific manner (Fig. 2A), with equivalent kinetics (Fig. 2B). The activity of PMA was inhibited by the selective inhibitor of PKC, bisindolylmaleimide II, indicating that enhancement of reporter gene expression by PMA is mediated through PKC activation (Fig.

2C). Gel shift assays revealed that TSA and PMA, but not HMBA, increased the binding activities for the NF- κ B consensus site (Fig. 2D). Since many transcription factors are substrates for histone acetyltransferases and protein kinases [31], it is plausible that TSA and PMA at least in part enhance the expression of a variety of reporter genes in 3T3 Swiss cells by inducing the binding activity for a *cis*-acting element as a result of the covalent modifications of transcription factors. Thus, reporter gene activation by TSA appears to be mediated through a combination of events, including hyperacetylation of core histones as well as transcription factors.

In this report, we have demonstrated that differentiation-inducing agents with diverse chemical structures and modes of action possess the ability to non-selectively enhance the expression of transiently transfected reporter genes. Thus, this property can serve as a biomarker for agents that cause leukemia cell differentiation. Because leukemia cell lines vary significantly in the sensitivity to chemical inducers of differentiation and no single leukemia cell line represents the general characteristics of all types of leukemia cells, it is impractical to select one leukemia cell line for screening of inducers of differentiation. Since the luciferase transfection assay is quick, easy, reproducible and cost-effective, it may be advantageous to use such a surrogate assay for the initial screening of differentiation inducers. In addition, the findings described in this report may have utility in the identification of unique targets that permit the development of novel agents for the attainment of the therapy of malignant diseases by terminal differentiation.

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Abbreviations

DMSO	dimethylsulfoxide
HMBA	hexamethylene bisacetamide
Hyp	hypoxanthine
TGua	6-thioguanine
TSA	trichostatin A
F-MEL	Friend murine erythroleukemia
HDAC	histone deacetylase
HAT	histone acetyltransferase
PMA	phorbol 12-myristate 13-acetate
PKC	protein kinase C
BII	bisindolylmaleimide II
HGPRT	hypoxanthine-guanine phosphoribosyltransferase
DIG	digoxigenin

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Fig. 1.

Stimulation of the expression of transiently transfected reporter genes in 3T3 Swiss cells by chemical inducers of differentiation. Cells were transfected with a reporter gene for 3 h and cultured in the absence or presence of chemical inducers for 20 h. Luciferase activities were normalized by cell number. Stimulation is expressed as the fold increase relative to the control value taken as 1.

Fig. 2.

Characterization of PMA as a stimulator of reporter gene expression. (A) Cells were transfected with one of six independent reporter genes and stimulation of the expression of the reporter gene by PMA was determined as in Fig. 1. (B) Cells were transfected with pNF-κB for 3 h and exposed to 50 nM PMA, 60 nM TSA, or 15 mM HMBA for the indicated times. (C) Cells were transfected with pNF-κB for 3 h and cultured in the simultaneous presence of inducer (10 nM PMA, 30 nM TSA, or 7.5 mM HMBA) and bisindolylmaleimide II (BII) at a concentration of 0.2 or 0.8 μM for 20 h. (D) Gel shift assay for DIG-labeled NF-κB and AP-1 consensus oligonucleotides incubated with nuclear extracts from 3T3 Swiss cells treated with inducers for 4 h.

Fig. 3.

Characterization of chemical inducers in histone modification and reporter gene expression. (A) Core histones were extracted from F-MEL cells treated with various inducers for 8 h, and the acetylation and phosphorylation status was determined by western analysis. Coomassie Brilliant Blue staining was used as a loading control. D₂ = diazepam. (B) 3T3 Swiss cells were transfected with the reporter HS2-421 β^m and treated with graded concentrations of TSA plus a fixed concentration of 7.5 mM HMBA (left panel), or graded concentrations of HMBA plus a fixed concentration of 15 nM TSA (right panel). (C) 3T3 Swiss cells were co-transfected with 0.2 μ g of both the reporter plasmid HS2-421 β^m and the expression plasmid encoding the

transcriptional modulator, and incubated with 20 nM TSA, 10 mM HMBA, or 50 nM PMA for 20 h.

Fig. 4.

Up- and down-modulation of endogenous genes by chemical inducers. (A) Total cell RNA from F-MEL cells treated with inducers for 8 h was subjected to northern hybridization (left panel). Total cell RNA from F-MEL cells exposed to HMBA or TSA for the indicated times was subjected to northern hybridization (right panel). (B) The acetylation status of core histones from F-MEL cells exposed to TSA for the indicated times was analyzed by Western blotting. The methylation status of histones was unaltered and served as a loading control.

Table 1

Differentiation of F-MEL cells by chemical inducers

Inducer	Concentration	Final cell density (cells/ml)	Benzidine positive cells (%)
F-MEL			
None	—	3.0×10^6	1
DMSO	1.5%	3.0×10^6	21
	2.0%	2.4×10^6	53
	2.5%	1.2×10^6	74
HMBA	2.5 mM	3.9×10^6	24
	5.0 mM	2.4×10^6	95
	7.5 mM	1.2×10^6	96
Hyp	2.5 mM	2.8×10^6	2
Diazepam	5.0 mM	2.4×10^6	26
	7.5 mM	1.2×10^6	35
	0.10 mM	2.7×10^6	27
	0.15 mM	2.4×10^6	64
	0.20 mM	0.83×10^6	82
HGPRT ⁻ F-MEL			
None	—	3.1×10^6	3
TGua	0.05 mM	2.2×10^6	30
	0.10 mM	1.7×10^6	59
	0.20 mM	1.4×10^6	83

Values represent triplicate averages of three independent experiments with standard errors less than 20%

Table 2

The promoter types in the reporter constructs used in these studies

Reporter gene	Promoter type
pGL3 control	SV40 promoter/enhancer
pNF- κ B-Luc	TATA box/5 $\times$ NF- κ B enhancer
p53-Luc	TATA box/15 $\times$ p53 enhancer
HS2-421 β^m	β^{major} -globin promoter/HS-2 ^a
pRL-TK	HSV thymidine kinase promoter
pRL-CMV	CMV immediate early promoter/enhancer

^aDNase I hypersensitive site 2 in murine β -globin locus control region.