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Phorbol ester treatment of HL 60 leukemia cells results in increase of β-(1→4)-galactosyltransferase

Valérie Pasqualetto [a], Stéphanie Lemaire [a], Dominique Neel [a], Michèle Aubery [a], Eric G. Berger [b], Christian Derappe [a,*]

[a] *Université de Paris V-René Descartes, UFR Biomédicale des Saints-Pères, 45, rue des Saints-Pères, F-75006 Paris, France*

[b] *Physiologisches Institut, Universität Zürich, Winterthurerstraße 190, CH-8057 Zurich, Switzerland*

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Abstract

We previously showed that HL 60 leukemia cells exhibit various changes in their cellular glycans after phorbol 12-myristate 13-acetate (PMA) treatment. These changes could originate largely from changes in one or several glycosyltransferases. In this report, we show using enzymatic measures, fluorescence microscopy, immunoblotting and Northern blot that β-(1→4)-galactosyltransferase I (GalT I) activity was higher ($> \times 2$) in PMA-treated compared with untreated HL 60 cells. Immunoblotting showed an increased intensity of the GalT I band at 49 kDa and Northern blot a weak increase of the GalT I transcript band, after PMA treatment. Moreover, Northern blot performed after actinomycin-D treatment of the cells, which inhibits transcription, suggests that the observed increase of GalT I expression could originate, in part, from increase of the stability of GalT I transcripts.

Keywords: HL 60 cells; Differentiation; Glycosylation; Galactosyltransferase

1. Introduction

The HL 60 cell line, derived from a patient with acute myeloid leukemia, has been widely used to investigate the process of myeloid differentiation, because these cells can differentiate into monocyte-like cells by treatment with phorbol 12-myristate 13-acetate (PMA) [1]. Differentiated HL 60 cells exhibited several morphological changes, including an increase of the size of the cell and a decrease of nuclear cytoplasmic ratio, and also many biological changes such as structural modifications of the carbohydrate chains expressed on cell glycoproteins [2–5]. More specifically, we observed [5] that PMA treatment of HL 60 cells resulted in lower expression of glycoproteins carrying complex-type carbohydrate chains. Conversely, they were enriched with non-reducing terminal *N*-acetylglucosamine residues. These glycans could possibly originate from down regulation of one or several glycosyltransferases involved in the biosynthesis of complex glycans. In fact, such changes in glycosyltransferase activities have previously been observed after HL 60 differentiation [6].

Among these glycosyltransferases, UDP-galactose:*N* - acetylglucosaminide - β - (1→4)-

* Corresponding author. Tel.: +33-1-42862089; fax: +33-1-42862080.

E-mail address: michele.aubery@biomedicale.univ-paris5.fr (M. Aubery).

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galactosyltransferases (GalT) constitute a family of glycosyltransferases responsible for the transfer of a galactose residue onto a *N*-acetylglucosamine residue. To date, seven members have been described, called GalT I to VII [7–10]. GalT I (EC 2.4.1.38) is a trans-Golgi resident type II membrane-bound glycoprotein involved in the biosynthesis of N- and O-linked carbohydrate moieties on glycoproteins and glycolipids [11]. According to species and cell types, various GalT I transcripts and glycoproteins have been described [12], and their genomic organization [13] and transcriptional regulation [14–16] have been extensively investigated.

In the present study, we have shown that activity and expression of GalT I in HL 60 cells increased after their treatment by PMA, partly attributable to an increase of stability of the GalT I transcripts.

2. Materials and methods

Chemicals.—Phorbol 12-myristate 13-acetate (PMA) and other chemicals were purchased from Sigma Chemical Co. (St Quentin-Fallavier, France).

Cell culture.—HL 60 cells were cultured in continuous suspension culture at the initial concentration of 3×10^5 cells/mL in T-75 plastic flasks (Falcon Laboratories, Grenoble, France) at 37 °C in a humidified atmosphere of 5% CO_2 in air, in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum and penicillin + streptomycin (5 IU/mL), all obtained from Life Technologies (Cergy-France). Cell viability, determined by trypan-blue-dye exclusion, was more than 95%. Cells were used between the 20th and 35th passages. They were induced to differentiate into monocyte-like cells by incubation in the presence of 50 ng/mL PMA for various times, as indicated.

GalT assays.—GalT assays were performed, as previously described [17], on extracts of 5×10^7 cells/assay using GlcNAc as substrate. [^{14}C]UDP-Galactose (UDP-Gal) (11.2 GBq/mmol, NEN Life Science Products, Le Blanc-Mesnil, France) was used at the specific activity of 40 Bq/nmol. Radioactivity was counted in a β-scintillation counter (Packard, Rungis, France). Radioactivity of assays performed without cellular extract and without exogenous acceptor was subtracted from the radioactivity of respective sample assays.

Immunoblot analysis.—Immunoblots were performed as previously described [17] on extracts of 2×10^8 cells. Glycoproteins were subjected to sodium dodecyl sulfate (SDS)–10% polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane. GalT was detected using a monoclonal anti-human GalT antibody (GT2/36/118) [18], followed by sheep anti-mouse Ig $F(ab')_2$ fragment coupled to horseradish peroxidase (Amersham-France, Les Ulis, France). Antibody-labeled proteins were stained with the 'Renaissance chemiluminescence reagent' (NEN Life Science Products).

Northern blot hybridization and stability assay for GalT I mRNA.—Total RNA was extracted according to Chomczynski and Sacchi [19] and subjected to electrophoresis in a 1.1% agarose gel as described by Thomas [20]. Two controls, λ-DNA *Hin*d III-digested and an RNA ladder (0.25–9.5 kb) were routinely included as size standards. One tenth of the treated RNA was run on a separate 1.1% agarose gel, which was then stained with ethidium bromide to control RNA integrity. After electrophoresis, gels were transferred onto Hybond N^+ filters (Amersham-France), prehybridized with salmon-sperm DNA and hybridized with [^{32}P]-labeled GalT I from HeLa cells [21] and human β-actin probes (Clontech Laboratories, Palo Alto, CA, USA), as internal standards.

To determine the relative stability of GalT I mRNA, cells were cultured for 24 h either in the presence or absence of PMA and incubated for an additional 4, 6 or 18 h in the presence of 10 μg/mL of actinomycin D, which inhibits transcription. Total RNA was extracted and analyzed by Northern blot hydridization using [^{32}P]-labeled GalT I and human β-actin probes. Radioactivity was quantitated using an automatic linear analyzer (Berthold, Rungis, France), relatively to the corresponding amount of β-actin.

Immunofluorescence microscopy.—Immunofluorescence microscopy experiments were performed as previously described [17], starting from 1×10^4 HL 60 cells and using the monoclonal antibody to GalT I (GT2/36/118) [18], followed by fluorescein isothiocyanate (FITC)-coupled sheep anti-mouse Ig (Amersham). After cytospin spreading on glass slides, cells were fixed, permeabilized and stained as described [17]. They were examined with a fluorescence microscope (Axiophot, Zeiss, Oberkochen, Germany) at a magnification $\times 640$.

3. Results and discussion

As shown in Table 1, PMA treatment of HL 60 cells resulted in a time-dependent increase of GalT activity. A more than two-fold increase was observed within 40 h, then, GalT activity continued to increase, but weakly (+ 8% until 78 h). Prolonged PMA treatment resulted in cell toxicity and cell death, as judged by trypan blue examination (data not shown), excluding the accurate measures of GalT activity. Among Gal-transferase, GalT I, GalT II and GalT III, are mainly expressed by circulating cells and are able to transfer galactose residues onto GlcNAc with a similar substrate specificity [7,8]. To obtain more information about the specific contribution of GalT I to the observed increase of GalT activity, immunoblotting and Northern blot have been performed using specific GalT I monoclonal antibody and cDNA probe, respectively.

Using the monoclonal antibody GT2/36/118 to GalT I from human milk [18], the expression of GalT I inside HL 60 cells was investigated by indirect immunofluorescence (Fig. 1). Only weak fluorescence was detected within untreated HL 60 cells, whereas after 48 h of PMA-treatment, a higher intensity of the fluorescence was observed. Moreover, it appears that in both untreated and PMA-treated HL 60 cells, GalT I was localized to the Golgi apparatus (Fig. 1 arrows).

Changes in GalT I expression were also detected by immunoblot analysis of cellular

Table 1
β-(1→4)-Galactosyltransferase activity measured in cellular extracts of untreated and phorbol ester-treated HL 60 cells [a]

Treatment	None	6 h PMA	24 h PMA	40 h PMA	48 h PMA	64 h PMA	72 h PMA	78 h PMA
GalT activity (nmol mg per h)	23.6 ± 0.6	18 ± 3.0	27.4 ± 2.4	43.4 ± 4.1	42.7 ± 3.0	49.2 ± 2.6	52.0 ± 2.6	53.5 ± 4.7

[a] Values are means ± S.D. of five independent experiments.

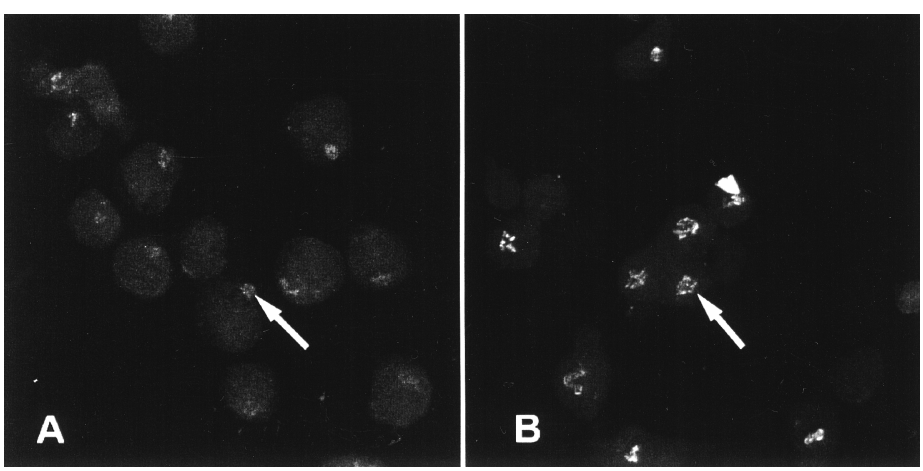

Fig. 1. Fluorescence microscopy of fixed and saponin-permeabilized HL 60 cells labeled with mAb directed against human GalT I and a sheep anti-mouse Ig-FITC-conjugated second antibody. (**A**) untreated HL 60 cells; (**B**) 48 h PMA-treated HL 60 cells. Magnification: $\times 640$.

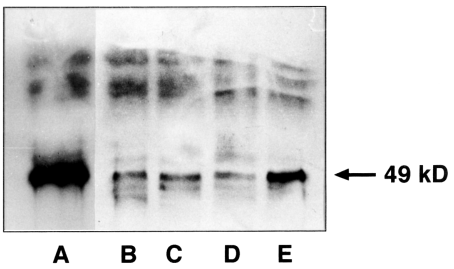

Fig. 2. Immunoblot analysis of GalT I performed on cell lysates of: (**A**) HeLa cells; (**B**) untreated HL 60 cells; (**C**) 6 h PMA-treated HL 60 cells; (**D**) 24 h PMA-treated HL 60 cells; (**E**) 48 h PMA-treated HL 60 cells.

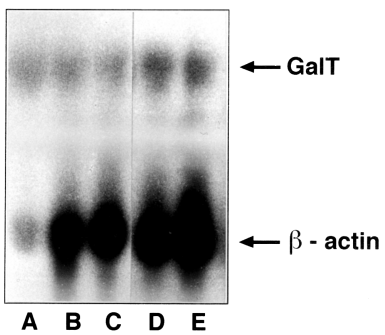

Fig. 3. Northern blot hydridization performed as described under Materials and Methods with 27 μg of total RNA from: (**A**) HeLa cells; (**B**) untreated HL 60 cells; (**C**) 6 h PMA-treated HL 60 cells; (**D**) 24 h PMA-treated HL 60 cells; (**E**) 36 h PMA-treated HL 60 cells.

extracts from untreated and PMA-treated HL 60 cells. As shown in Fig. 2, GalT I appeared as a broad band at 49 kDa in all samples, as previously reported for human GalT I [22]. This 49 kDa band appeared at the same position as compared with the GalT I band from HeLa cells. Other GalT could also be present in HL 60 cells, especially GalT II, and the possibility of cross reaction with our GalT I monoclonal antibody should be considered. However, this monoclonal antibody did not result in cross reaction with the bovine GalT I, which has 78% homology with the human GalT I, thus, cross reaction between our monoclonal antibody and Gal T II, which has only 55% homology with GalT I, appears unlikely. This was strengthened by another observation: the GalT I monoclonal antibody that we used was completely absorbed with purified GalT I from human milk (E.G. Berger, personal communication). Immunoblotting (Fig. 2) showed that 6 h of PMA treatment did not induce significant changes in expression of the 49 kDa band, whereas 24 and 48 h of PMA treatment resulted in stronger intensity of the GalT I band. These results parallel GalT activity, which required a lapse of almost 2 days of PMA treatment and suggest that the changes in GalT activity reflect increasing amounts of the GalT protein rather than changes of the enzyme's activation state.

Northern blot analysis, performed using a cDNA probe prepared from HeLa cells [21] and corresponding to the GalT I [9], showed (Fig. 3) that GalT I transcripts form a single band of 4.2 kb, as observed for most human cells. Although 6 h of incubation with PMA had no appreciable effect on this band, 24 and 36 h of incubation showed a weak increase in intensity of the GalT I transcript band. This increase was confirmed by quantitation of radioactivity, since an increase of 50% was measured using a linear analyzer of radioactivity. Prolonged PMA treatment until 48 h did not increase the intensity of the GalT I band (data not show).

The increasing amounts of GalT I transcripts could result either from enhanced transcription of the *GalT I* gene or an enhanced stability of transcripts or both. To determine whether or not PMA treatment increased the mRNA stability, we prevented gene transcription by adding actinomycin D and measured the degradation of GalT mRNA over time relative to that of the standard mRNA (β-actin). As shown in Fig. 4, PMA treatment led to a weak increase of stability of GalT mRNA.

Thus, the increase in the number of galactose residues on non-reducing terminal GlcNAc residues of complex-type carbohydrate chains that we previously observed after PMA treatment of HL 60 cells [5] could be, for a part, ascribed to a lower level of GalT I activity in untreated as compared with in PMA-treated HL 60 cells. Changes in stability of GalT I mRNA seem to be involved, but exact molecular mechanisms remain unknown. This increase could be either in relation to monocytic differentiation of HL 60 cells or due to a specific action of PMA, since this treatment triggered an activation cascade involving successively in the initial steps, phospholipase D and subunits β_I and β_{II} of

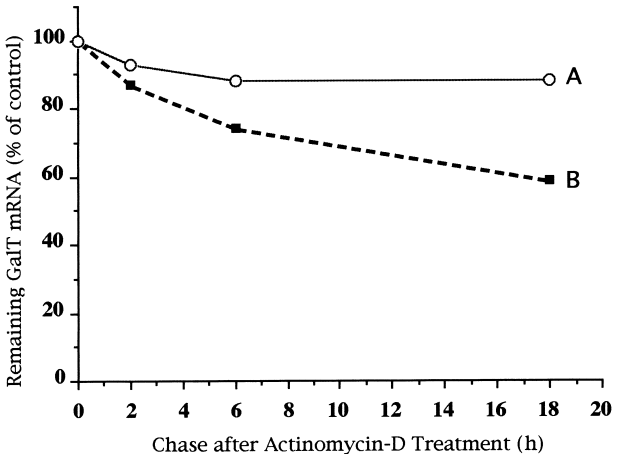

Fig. 4. Stability of GalT I mRNA in HL 60 cells on PMA treatment. Cells were cultured with 50 ng/mL PMA (**A**) or without PMA (**B**) for 24 h, followed by addition of 10 μg/mL of actinomycin D. GalT I mRNA and GAPDH mRNA levels were measured from 2 to 18 h, as described under Material and Methods and plotted. Results give the remaining GalT I mRNA in percentages of control. They are mean values of two experiments with similar results.

PKC [23–25], possibly able to increase activity of GalT I.

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