AP-1 binding activity is modulated by post-translational modifications during granulocytic differentiation of HL-60 cells

J. Savickienė*,
A. Pivoriūnas,
G. Treigytė,
R. Navakauskienė

Department of Developmental Biology, Institute of Biochemistry, Vilnius, Lithuania

Activating protein (AP-1) transcription factors participate in the regulation of different target genes and thus execute distinct biological functions such as cell proliferation, differentiation and apoptosis. The present study examined the influence of post-translational modifications (phosphorylation, acetylation, glycosylation) on the activation of AP-1 in the human promyelocytic leukemia HL-60 cells. We have shown that the binding properties of AP-1 to promoter regions of myeloid genes gradually increased during granulocytic HL-60 cell differentiation induced by retinoic acid (RA). Hyperacetylation and the inhibition of N-glycosylation or tyrosine phosphorylation up-regulated AP-1 binding to DNA at the commitment stage for differentiation. Blocking of serine-threonine kinase PKC increased AP-1 binding activity during the cell maturation only. Post-translational modifications had a marked influence on HL-60 cell fate and thus could regulate myeloid genes by involvement of AP-1.

Key words: transcription factors, leukemia, differentiation

INTRODUCTION

Leukemias are often associated with defects in differentiation resulting in an abnormal accumulation of immature blasts [1]. The factors that regulate these events have not been completely elucidated but include diverse nuclear regulators (transcription factors). Post-translational modifications of proteins adds a further level of regulation, enlarging the protein capacity to take part in regulation events. The AP-1 (activating protein-1) family of transcription factors consists of homodimers or heterodimers of Jun, Fos or ATF that bind to a common DNA site, the AP-1 binding site [2]. Stimulation of cells with diverse stimuli induces the members of this family differently [3]. There is evidence that the activity of AP-1 components is involved in differentiation of myeloid cells [7]. We show here that post-translational modifications may modulate granulocytic HL-60 cell differentiation through involvement of AP-1 activity.

* Corresponding author: Department of Developmental Biology, Institute of Biochemistry, Mokslininkų 12, LT-2600 Vilnius, Lithuania. Fax: +370-5 2729196. E-mail: savickiene@bchi.lt

MATERIALS AND METHODS

Cell culture. Human promyelocytic HL-60 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified 5% CO_2 atmosphere at 37 °C. The degree of differentiation was assayed by the ability of cells to reduce nitro blue tetrazolium (NBT) after stimulation with PMA [5].

Electrophoretic mobility shift assays (EMSA). Nuclear extracts from exponentially growing HL-60 cells and induced to differentiate by 1 μM RA were prepared according to previously described methods [6]. EMSAs were performed by incubating 10 μg nuclear extracts with the double-stranded oligonucleotide containing consensus binding sites for AP-1 labelled with [γ-^{32}P-ATP] by using T4 polynucleotide kinase as previously described [7].

RESULTS AND DISCUSSION

As is shown in Fig. 1A, HL-60 cell differentiation was associated with a gradual increase in nuclear protein binding to the AP-1 oligonucleotide, which starts 30 min following RA- treatment. The specificity of complexes was shown by disappearance of

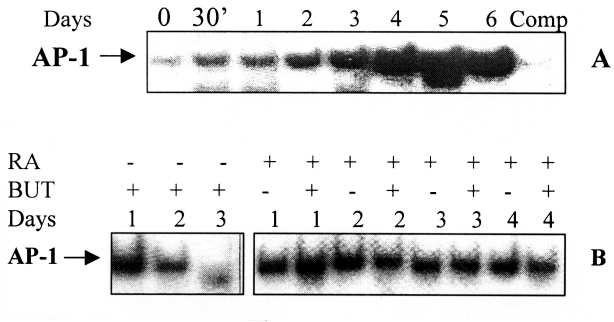

Fig. 1. AP-1-binding activity in HL-60 cells exposed to RA or sodium butyrate during granulocytic differentiation. Nuclear extracts were prepared from HL-60 cells induced by RA for 6 days (A) or from proliferating (first panel) and differentiating (second panel) cells exposed to histone deacetylase inhibitor But (B) for 3 and 4 days, respectively. EMSAs were performed as described in Methods

the AP-1 band in competion assay using unlabeled AP-1 oligonucleotide at a 50-fold excess (Fig. 1, comp). The finding indicates that RA specifically activates AP-1 binding to myeloid promoters.

To examine the influence of deregulated phosphorylation on AP-1 activity, RA-induced cells were exposed to inhibitors: of tyrosine protein kinases, 25 μM lavendustin C (Lav), or tyrosine phosphatases, 100 μM sodium vanadate (Van), or serine-threonine kinase PKC, 100 nM calphostin C. Lav inhibited RA-induced HL-60 cell differentiation about two-fold (Fig. 2). Van caused a cell death at day 3 (data not shown). In proliferating HL-60 cells, Van did not change AP-1 binding at 24 h of exposure, while concomitant treatment with Van and RA during 48 h caused a sustained AP-1 activation (Fig. 3A). Treatment with Lav of uninduced or RA-induced HL-60 cells was associated with a significant increase in the intensity of the more slowly migrating band at 24 h (Fig. 3), which was associated with a block for induction of differentiation. Exposure to RA and Clp synergistically enhanced RA-induced HL-60 cell differentiation (150% of control) (Fig. 2). AP-1 binding activity decreased following treatment with Clp during the first 24 h and markedly increased during the cell maturation from day 3 (Fig. 3B). In understanding how acetylation may alter interactions between AP-1 and DNA, HL-60 cells were incubated with the inhibitor of histone deacetylase, 250 nM sodium butyrate (But) in the presence or absence of RA. The But alone induced cell differentiation at the same level as RA and two-fold enhanced it in combination with RA (Fig. 2). It caused a marked response to AP1-DNA binding in proliferating cells during 24 h of treatment, which decreased during the next 24 h and disappeared at day 3 (Fig. 1B). In differentiating cells, the formation of AP-1-DNA complexes was upregulated only on the first day of simultaneous exposure to But and RA. To investigate a link between glycosylation and transcriptional regulation of AP-1, HL-60 cells were incubated with RA and the inhibitor of N-glycosylation, 1 μM tunicamycin (Tun) or inhibitor of O-glycosylation, 2 mM IPTG. IPTG drastically inhibited the RA-induced HL-60 differentiation, while Tun increased it by 35% (Fig. 2). The formation of complexes by Tun was associated positively with the commitment for differentiation

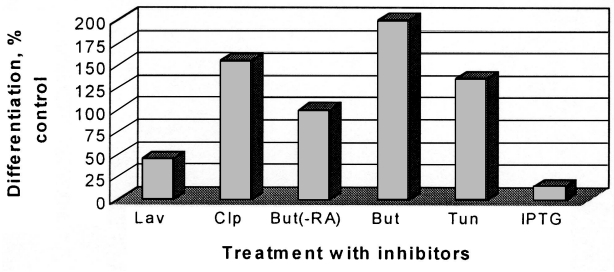

Fig. 2. Effects of post-translational modifications on HL-60 cell differentiation. HL-60 cells induced by RA were treated for 5 days with specific inhibitors of phosphorylation (Lav, Van, Clp), glycosylation (Tun, IPTG) or histone deacetylases (But). Cell differentiation was determined by NBT reducing ability on day 5

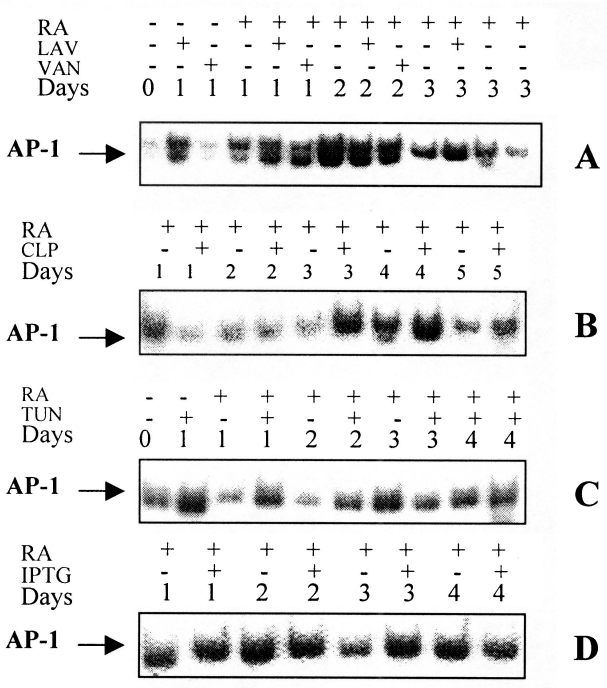

Fig. 3. Modulation of AP-1 binding activity by inhibitors of phosphorylation and glycosylation. HL-60 cells induced by RA were treated for the indicated time with specific inhibitors of phosphorylation: Lav or Van (A), Clp (B); or glycosylation: Tun (C) or IPTG (D). EMSAs were performed as described in Methods

(Fig. 3C). The pattern of AP-1 binding during differentiation did not change after treatment with IPTG (Fig. 3D).

The results obtained show that AP-1 binding intensity caused by hyperacetylation and O-glycosylation, contrary to tyrosine dephosphorylation, is associated with a signal for granulocytic differentiation, while the influence of PKC-dependent phosphorylation on AP-1 activity is maturation-related.

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AP-1 IR DNR SĄRYŠIO AKTYVUMĄ MODULIUOJANČIOS POSTTRANSLIACINĖS MODIFIKACIJOS HL-60 LĄSTELIŲ GRANULIOCITINĖS DIFERENCIACIJOS METU

S a n t r a u k a

Aktyvuojančio baltymo (AP-1) transkripcijos veiksniai reguliuoja skirtingos biologinės funkcijos genus, dalyvaujančius ląstelių proliferacijoje, diferenciacijoje ir apoptozėje. Šiame darbe buvo tiriamas posttransliacinių modifikacijų (fosforilinimo, acetilinimo, glikozilinimo) poveikis AP-1 aktyvumui žmogaus promielocitinės leukemijos ląstelėse HL-60. Nustatyta, kad AP-1 sąryšio efektyvumas mieloidinių genų promotorinėms sritims palaipsniui didėja retinoine rūgštimi indukuotos granuliocitinės diferenciacijos metu. Diferenciaciją lemiančioje stadijoje dėl hiperacetilinimo ir N-glikozilinimo bei tirozino fosforilinimo inhibavimo padidėja AP-1 sąryšio su DNR intensyvumas. Blokuojant serino-treonino kinazę PKC AP-1 sąryšio aktyvumas sustiprėja tik bręstant granuliocitams. Taigi posttransliacinės modifikacijos pakeičia HL-60 ląstelių likimą, todėl manoma, kad jos gali reguliuoti mieloidinių genų veiklą įtraukiant AP-1.