Transcriptional profiles of histone acetyltransferases in human monoblast U937 cells treated with all-*trans* retinoic acid or 1α, 25-dihydroxyvitamin D_3

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(Accepted Feb. 8. 2021)

要旨：ヒストンアセチルトランスフェラーゼ（HAT）は、コアヒストンのアセチル化を触媒する酵素である。ヒストンアセチル化はDNAに対するコアヒストンの親和性を減弱し、転写を活性化させる。この現象は、真核生物のクロマチン構造の変化を介した転写調節において重要な役割を担っている。HATはホモロジーと機能に基づいてGNATファミリー（GCN5, PCAF, HAT1, Elp3及びATF-2）、MYSTファミリー（Tip60, MORF及びMOZ）、p300/CBP、SRC及びClockといった幾つかのクラスに分類される。この論文では、脂溶性ビタミン類によって誘導されるマクロファージ分化におけるHAT遺伝子の転写プロファイルを得るために、レチノイン酸または活性型ビタミン D_3 でマクロファージ様細胞に分化誘導したヒト単芽球様株細胞U937でのHAT遺伝子の転写レベルの変化を半定量性RT-PCR法で調べた。その結果、特にレチノイン酸処理によって、PCAF, MORF, SRC1及びACTRの転写が顕著に増強されることがわかった。これらの結果は、脂溶性ビタミン類によって誘導されるマクロファージ分化のエピジェネティック制御機構の解明に寄与するものと期待できる。

キーワード：ヒストンアセチルトランスフェラーゼ、マクロファージ、分化、転写、脂溶性ビタミン

Summary

Histone acetyltransferases (HATs) are involved in the acetylation of core histones, resulting in the attenuated affinity of core histones to DNA. This is an important event for transcriptional regulation via alterations in the chromatin structure in eukaryotes. HATs are divided into several classes based on sequence homology and function, such as GNAT (GCN5, PCAF, HAT1, Elp3 and ATF-2), MYST (Tip60, MORF and MOZ), p300/CBP, SRC and Clock. In this paper, to obtain the transcriptional profiles of various HAT genes during fat-soluble vitamins-induced macrophage development, we carried out semiquantitative RT-PCR using total RNA prepared from all-*trans* retinoic acid (RA) or 1α, 25-dihydroxyvitamin D_3 (VD)-treated human monoblast U937 cells. Semiquantitative RT-PCR revealed that RA causes remarkable enhancement of transcription of PCAF, MORF, SRC1 and ACTR compared to VD. Our data will contribute to elucidation of the epigenetic mechanisms of fat-soluble vitamins-induced macrophage differentiation.

Key words: histone acetyltransferase, macrophage, differentiation, transcription, Fat-soluble vitamins

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Introduction

Macrophages ubiquitously exist in both vertebrates and invertebrates. They play key roles in not only innate immune system by killing various invading microorganisms but also acquired immune system by presenting digests of invading microorganisms as antigens to helper T cells. Macrophages develop from the hematopoietic stem cells via common myeloid precursor cells followed by monocytes[1), 2)]. To understand immune system, it is very important to know how macrophages differentiate in peripheral tissue[3)]. However, little is known regarding mechanisms of macrophage differentiation. In particular, the mechanisms of epigenetic controls such as histone acetylation in macrophage differentiation remain to be resolved.

Histone acetylation catalyzed by histone acetyltransferases (HATs) is an important reaction for transcriptional regulation through alterations in the chromatin structure[4)~6)]. HATs transfer the acetyl group from acetyl-CoA to ε-amino groups of lysine residues of core histone. The acetylation occurs neutralization of positive charges of core histones, resulting in the attenuated affinity of core histones to DNA. HATs are divided into several classes based on sequence homology and function, such as GNAT (Gcn5-related N-acetyltransferase; GCN5, PCAF, HAT1, Elp3 and ATF-2), MYST (Tip60, MORF and MOZ), p300/CBP (CREB-binding protein), SRC (steroid receptor coactivator; SRC1 and ACTR) and Clock[7), 8)]. To elucidate the mechanisms of macrophage differentiation, it is necessary to know which HATs are involved in regulation of development of macrophages.

In this paper, to obtain the typical transcriptional profiles of various HAT genes during macrophage development, we carried out semiquantitative RT-PCR using total RNA prepared from all-*trans* retinoic acid (RA) or 1α, 25-dihydroxyvitamin D_3 (VD)-treated human monoblast U937 cells.

Materials and Methods

1. Materials

RA, VD and luminol (Sigma, MO, USA), Diogenes (National Diagnostics, GA, USA), RPMI-1640 culture medium (Gibco Laboratories, MD, USA), phorbol 12-myristate 13-acetate (PMA) (Calbiochem., Darmstadt, Germany), fetal bovine serum (FBS) (JRH Biosciences, KS, USA) and plasmocin (InvivoGene, CA, USA) were obtained.

2. Cell culture and treatment with RA or VD

Human monoblastic leukemia U937 cells (RCB0435) were provided by the RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan. Cells were grown in RPMI-1640 culture medium containing 10% FBS and 5 mg/mL plasmocin as described[9)]. Cells (1.0 x 10^6) in 5 ml of culture medium were incubated in the absence or presence of 1 μM RA or 100 nM VD at 37°C for 48 h.

3. Assay of superoxide (O_2^-) generation

O_2^- generation was quantified by measuring chemiluminescence (CL) using Diogenes-luminol CL probes[10)]. Cells (1.0 x 10^6 cells/mL) were resuspended in PBS containing 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 5 mM glucose and 0.03% bovine serum albumin were stimulated with 200 ng/mL PMA at 37°C for 10 min. O_2^- generation was measured by Lumat^3 LB9508 luminometer (Berthold Technologies, Bad Wildbad, Germany).

4. Semiquantitative RT-PCR

Total RNA samples were isolated by the cells with Trizol reagent (Invitrogen, CA, USA). Semiquantitative RT-PCR was performed as described using specific sense and antisense primers of human HATs gene (Table 1)[4), 10)]. Human GAPDH gene was used as internal controls. PCR products were subjected to 1.5% agarose gel electrophoresis. Data obtained before reaching the plateau were analyzed by Quant-AMZ software (Totallab., Newcastle upon Tyne, UK) using a luminescent image analyzer STAGE-5100 (AMZ System Science, Osaka, Japan).

Table 1: A list of oligonucleotides used as primers for RT-PCR

	Forward primers	Reverse primers
GCN5	AACCTGCTGGCCCAAATCAA	CTACTTGTCAATGAGGCCTC
PCAF	AGAAACTCGGAGTGTACTCC	GCCTTCAACCACAGGTTTTC
HAT1	TGGCGGGATTTGGTGCTATG	TCATCTGCCTCTACACAGTC
Elp3	ATGAGGCAGAAGCGGAAAGG	ACCTTGCGATACTGAGGAGG
ATF-2	AGGCAATACAAGGACCTGTG	GAGGGGATAAATCTAGAGGC
Tip60	ATCCTGAGCGTGAAGGACAT	ATCCTGAGCGTGAAGGACAT
MORF	TCTCTGAACAGCTGGAACTC	CTGGGTTGTTGGTGGTGCTT
MOZ	CAGAAACAGCGTCCTTCAGA	CAGACACATCCTTCTGACCT
p300	ATGGCCGAGAATGTGGTGGA	CACCAACTCCCATATTGAGG
CBP	ATGGCTGAGAACTTGCTGGA	CATGTTAGCACTGTTCGGCT
SRC1	CTGCTAACCCAGACTCACAT	CTGCTAACCCAGACTCACAT
ACTR	AAACTTGGATCCACTGGCCA	TCCCTGCCCTGTAGAAGATA
Clock	GATGGGTTGGTGGAAGAAGA	CACTAGCATCTGACTGTGCA
GAPDH	ATGGGGAAGGTGAAGGTCGG	GGGTGGAATCATATTGGAACATGTAAAC

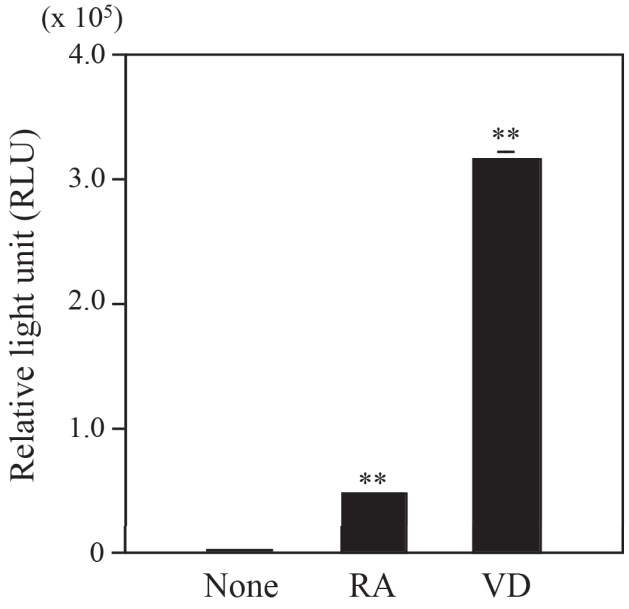

Fig. 1. PMA-induced O_2^- generation by U937 cells cultured with RA or VD. Cells (1.0 x 10^6) in 5 ml of culture medium were incubated without (None) or with 1 μM RA or 100 nM VD at 37°C for 48 h. The cells were stimulated with 200 ng/mL PMA and their CL responses were determined by a luminometer as described in "Materials and Methods". Data represent the averages of three separate experiments. Error bars indicate standard deviation. Statistical differences were calculated using Student's *t* test. **, $p < 0.01$ compared with the data of "None".

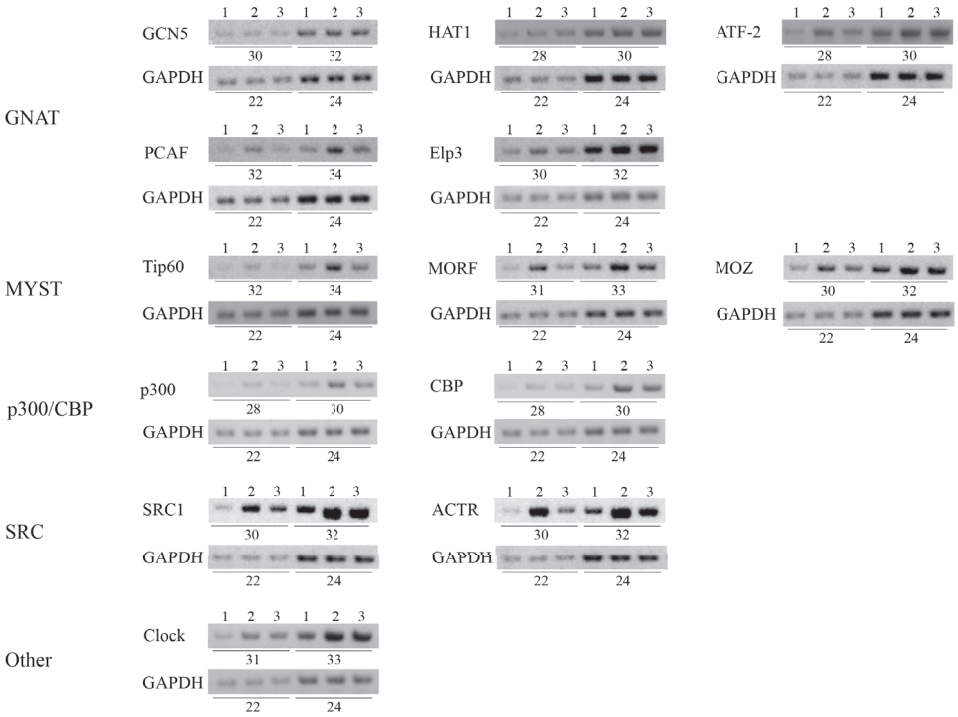

Fig. 2. Typical semiquantitative RT-PCR profiles of HAT genes (lane 1: untreated U937 cells, lane 2: RA-treated U937 cells, lane 3: VD-treated U937 cells). Cycle numbers of PCR are shown below the panels.

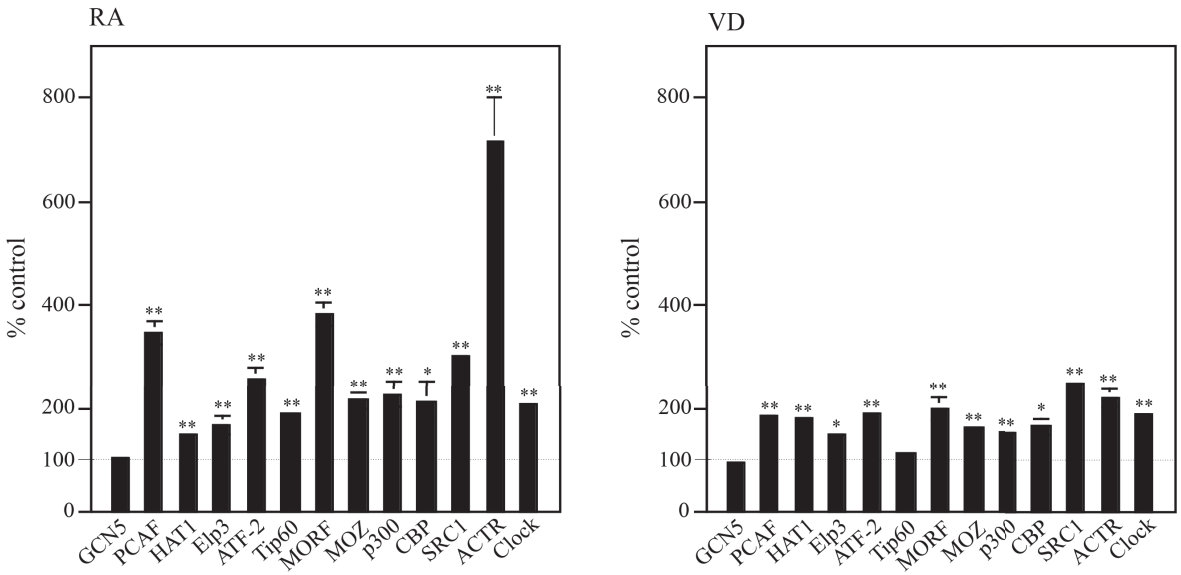

Fig. 3. Influences of RA or VD on transcription of HAT genes. Cells (1.0×10^6) in 5 ml of culture medium were incubated without or with 1 μM RA or 100 nM VD at 37°C for 48 h. The mRNA levels of HATs were determined by semiquantitative RT-PCR using total RNAs extracted from untreated, RA-treated and VD-treated U937 cells. Data calibrated with the internal controls (human GAPDH) are indicated as percentages of control values obtained from untreated U937 cells, and represent the averages of three separate experiments. Error bars indicate standard deviation. Statistical differences were calculated using Student's *t* test. *, $p < 0.05$; **, $p < 0.01$ compared with the data of untreated U937 cells.

5. Statistical analysis

Data (O_2^- and RT-PCR) obtained are presented as averages of three separate and independent experiments. Error bars indicate standard deviation. Statistical differences were calculated with Student's *t* test.

Results and Discussion

The human monoblast U937 cells are known to differentiate to macrophage-like cells in response to various agents such as porbol esters, interferon-γ, fat-soluble vitamins (RA, VD) and so on, resulting in enhancement of O_2^- generation[11), 12)]. Without RA or VD treatment, U937 cells generated a negligible level of O_2^-. After RA or VD treatment, in contrast, the O_2^--generating activities were dramatically enhanced (Fig. 1). These data showed that treatment with RA or VD certainly causes differentiation of U937 cells.

Next, to know the effects of RA or VD on transcription of various HAT genes that are thought to play especially important roles in transcriptional regulation, we carried out semiquantitative RT-PCR on total RNA prepared from untreated, RA-treated, and VD-treated U937 cells. Typical semiquantitative RT-PCR profiles are shown in Fig. 2. Quantitative data of semiquantitative RT-PCR were indicated as percentages of control values obtained from untreated U937 cells (Fig. 3). In RA-treated U937 cells, transcription of PCAF, HAT1, Elp3, ATF-2, Tip60, MORF, MOZ, p300, CBP, SRC1, ACTR and Clock were significantly increased (to ~350%, ~150%, ~170%, ~260%, ~190%, ~390%, ~220%, ~230%, ~220%, ~300%, 720% and ~210%, respectively), while that of GCN5 did not change. On the other hand, in VD-treated U937 cells, transcription of PCAF, HAT1, Elp3, ATF-2, MORF, MOZ, p300, CBP, SRC1, ACTR and Clock were certainly increased (to ~190%, ~180%, ~150%, ~190%, ~200%, ~170%, ~160%, ~170%, ~250%, 230% and ~190%, respectively), while those of GCN5 and Tip60 did not change. These results revealed that treatment with RA or VD enhances transcription of most of the HATs tested.

In this paper, we show that treatment with RA or VD of U937 cells brought about enhancement of transcription of many HAT genes during differentiation to macrophage-like cells. However, RA caused remarkable enhancement of transcription of PCAF, MORF, SRC1 and ACTR compared to VD, although induction of O_2^- generation by RA was remarkably weaker than that by VD. This discrepancy cannot be still explained. In addition, up-regulation of transcription cannot always bring about enhancement of amounts and activities of proteins. To know which HATs participate in regulation of fat-soluble vitamins-induced differentiation of macrophages, further research will be necessary in the future. However, we believe that our data in this paper will contribute to elucidation of the epigenetic mechanisms of macrophage differentiation.

Acknowledgements

This work was supported in part by JSPS KAKENHI Grant Number 19K02329 (to H. K.).

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