Biochemical and Biophysical Research Communications 458 (2015) 180–185

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Glucocorticoids facilitate the transcription from the human cytomegalovirus major immediate early promoter in glucocorticoid receptor- and nuclear factor-I-like protein-dependent manner

Maki Inoue-Toyoda [a, 1], Kohsuke Kato [a, b, 1], Kyosuke Nagata [c, *], Hiroyuki Yoshikawa [a, b]

[a] Graduate School of Comprehensive Human Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8575, Japan
[b] Faculty of Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8575, Japan
[c] University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8575, Japan

ARTICLE INFO

Article history:
Received 25 December 2014
Available online 29 January 2015

Keywords:
Human cytomegalovirus (HCMV)
Major immediate early (MIE) promoter
Glucocorticoid
Glucocorticoid receptor (GR)
Dexamethasone (DEX)
Nuclear factor-I (NF-I)

ABSTRACT

Human cytomegalovirus (HCMV) is a common and usually asymptomatic virus agent in healthy individuals. Initiation of HCMV productive infection depends on expression of the major immediate early (MIE) genes. The transcription of HCMV MIE genes is regulated by a diverse set of transcription factors. It was previously reported that productive HCMV infection is triggered probably by elevation of the plasma hydroxycorticoid level. However, it is poorly understood whether the transcription of MIE genes is directly regulated by glucocorticoid. Here, we found that the dexamethasone (DEX), a synthetic glucocorticoid, facilitates the transcription of HCMV MIE genes through the MIE promoter and enhancer in a glucocorticoid receptor (GR)-dependent manner. By competitive EMSA and reporter assays, we revealed that an NF-I like protein is involved in DEX-mediated transcriptional activation of the MIE promoter. Thus, this study supports a notion that the increased level of hydroxycorticoid in the third trimester of pregnancy reactivates HCMV virus production from the latent state.

1. Introduction

Human cytomegalovirus (HCMV), a member of herpesvirus, is common and usually asymptomatic in healthy children and adults including pregnant women. Initiation of HCMV productive infection is dependent on the expression of the major immediate early (MIE) genes. The virus establishes a lifelong latent infection and is periodically reactivated from latency, producing infectious viruses [1]. Congenital HCMV infection, which occurs *in utero* via the placenta, may cause fatal and neonatal death, developmental defects, or serious clinical sequelae. It is reported that the virus is reactivated more often in the third trimester than in the second or the first of pregnant women [2]. In this reactivation process, the transcriptional regulation of the MIE genes is one of the most important key events for both HCMV life cycle and clinical results.

The transcription of the MIE genes is driven by the MIE regulatory region encompassing approximately 2 kbp region upstream of the transcription start site including a core promoter, enhancer, unique region, and distal modulator [3]. The MIE regulatory region contains binding sites for a diverse set of cellular transcription factors [3–8]. Adrenal glucocorticoid hormones such as cortisol are known to play significant roles in the regulation of inflammatory signals thorough gene regulation [9]. The glucocorticoid-bound glucocorticoid receptor (GR) binds to its response element (GRE) and regulates transcription. It was previously reported that the production of HCMV progeny viruses was facilitated in dexamethasone (DEX)-treated cells but not in other hormones-treated cells [10]. The expression level of MIE proteins was increased by DEX treatment in HCMV-infected cells. These results suggested that DEX treatment facilitates HCMV virus production through activation of the MIE gene expression. Recently, it was reported that glucocorticoids trigger reactivation of HCMV from HCMV-latently infected myeloid cells and increases the incidence of HCMV infection in liver transplant patients when both donor and recipient are HCMV seropositive [11]. Furthermore, it is suggested that HCMV infection in the third trimester of pregnant women is probably due to elevation of the plasma hydroxycorticoid level [12]. However, it has never been clarified whether DEX-mediated signal pathway is directly involved in the transcriptional regulation of the MIE genes.

* Corresponding author. Fax: +81 29 853 3942.
E-mail address: knagata@md.tsukuba.ac.jp (K. Nagata).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2015.01.091
0006-291X/

Here, we found that the DEX treatment facilitates the transcription of the HCMV MIE genes through the MIE promoter and enhancer region in a GR-dependent manner. By competitive EMSA and reporter assays, we revealed that an NF-I-like protein is involved in the DEX-mediated transcriptional activation of the MIE promoter.

2. Materials and methods

2.1. Cell culture, virus preparation, and infection

BJ-TERT cells were maintained in a mixed medium (4:1) of Dulbecco modified Eagle medium (DMEM, Nissui) and Medium 199 (Gibco-Life technology) (DMEM-Medium 199) containing 10% (v/v) fetal bovine serum (FBS) and 1 mM sodium pyruvate. HeLa cells were maintained in DMEM containing 10% (v/v) FBS. Infection experiments and propagation of HCMV AD169 strain were basically performed as follows: BJ-TERT cells in DMEM-Medium 199 mixed medium containing 2% FBS were infected with AD169 strain at appropriate infectious units. After incubation for 1 h at 37 °C, DMEM-Medium 199 containing 10% FBS was added and further incubated for desired periods. Infectious units of AD169 strain were determined by indirect immunofluorescent analyses with anti-HCMV IE72 antibody (Millipore) using BJ-TERT cells.

2.2. Plasmid transfection and luciferase assay

HeLa cells were transfected with pMIEP-Luc reporter plasmid using GeneJuice® (Novagen) in combination with a control plasmid DNA expressing secreted alkali phosphatase (SEAP). At 24 h after transfection, the cell culture medium was collected, and the SEAP activity was measured using SEAP assay kit (TOYOBO) for evaluating the transfection efficiency. At the same time, the culture medium was exchanged with fresh one containing desired concentrations of DEX and/or RU486, one of GR antagonists, and cells were further incubated for 24 h. Cells were washed with PBS and lysed in a cell lysis buffer (25 mM Tris–HCl [pH 7.9], 10% glycerol, and 0.1% TritonX-100) by three freezing–thawing cycles. The cell lysates and a luciferase substrate (Promega) were mixed, and the luciferase activity was measured by Lumat LB9506 (BERTHOLD). Data were normalized by the SEAP activity.

2.3. Reverse transcription and quantitative PCR analyses

Total RNA was prepared from cells using RNeasyR Mini Kit (QIAGEN) in combination with DNaseI. The concentration of RNA in each sample was determined using a Nano Drop Lite spectrophotometer (Thermo Scientific). To analyze the level of the MIE gene and *β-actin* mRNAs, cDNA was synthesized from the total RNA prepared from AD169-infected BJ-TERT cells using RevaTra Ace reverse transcriptase (TOYOBO) and oligo-dT primer. To analyze the level of mature and pre-mature *luciferase* mRNAs, cDNAs were synthesized from total RNA prepared from pMIEP-Luc-509 plasmid DNA-transfected HeLa cells using RevaTra Ace reverse transcriptase and oligo-dT primer or a reverse primer corresponding to complementary sequence within the intron located at immediately upstream of SV40 polyA signal in PGV-B plasmid, 5′-TCAGTAGTTTAACACATTATACACTT-3′ (R2). The primer sets used for quantitative PCR (Q-PCR) were as follows: for *MIE IE72*, 5′-CCTAGTGGATGACCTA-3′ and 5′-GTGACACCAGAGAATCAG-3′; for *β-actin*, 5′-ATGGGTCAGAAGGATTCCTATGT-3′ and 5′-GGTCATCTTCTCGCGGTT-3′; for *GAPDH*, 5′-AGCCAAAAGGGTCATCATCTC-3′ and 5′-GGACTGTGGTCATGAGTCCTTC-3′; for mature *luciferase* (*Luc*), 5′-ACTGCGATTTTAAGTGTTGTTCCAT-3′ (F1) and 5′-GTGCGCCCCCAGAAGCAATTTC-3′ (R1); and for premature *luciferase* (pre*Luc*), 5′-GGGCGGAAAGTCCAAATTGT-3′ (F2) and R2 primer. Q-PCR reactions were performed with FastStart SYBR Green Master (Roche) using Thermal Cycler Dice (Takara).

2.4. Preparation of nuclear extracts and electrophoretic gel mobility shift assay (EMSA)

HeLa cells were treated with or without 1 μM DEX for 24 h at 37 °C. Nuclear extracts were prepared as described previously [13]. Total protein concentration of nuclear extracts was determined by the Bradford method. The 21 bp MIEP probe was obtained by annealing oligonucleotides 5′-GGTTTTGGCAGTACATCAATG-3′ and 5′-CATTGATGTACTGCCAAAACC-3′. Radioactive end-labeling was performed using T4 polynucleotide kinase and [γ-^{32}P]ATP. For competition assays, competitor DNAs were generated by annealing the following oligonuceotides: for SV40 21 bp repeat, 5′-CATTCTCCGCCCCATGGC-3′ and 5′-GCCATGGGGCGGAGAATG-3′; for NF-I, 5′-CGATCTGGCACTGTGCCAAGC-3′ and 5′-GCTTGGCACAGTGCCAGATCG-3′; and for mutated MIEP, 5′-GGTTTAGCGAGTACATCAATG-3′ and 5′-CATTGATGTACTCGCTAAACC-3′. DNA binding reactions were performed in 10 μl reaction mixture containing 20 mM Hepes-NaOH (pH7.9), 1 mM EDTA, 50 mM KCl, 6.25 mM $MgCl_2$, 1 mM DTT, 0.5 mM PMSF, 0.2 mg/ml BSA, 10% Glycerol, 4 μg poly (dI-dC). Nuclear extracts (10 μg protein) were incubated with radioactively labeled MIEP oligonucleotide probes (0.01 pmol) in the presence or absence of MIE, SV40, NF-I, or mutated MIE competitor DNAs (0.3 or 1 pmol) at 30 °C for 15 min. Reaction mixtures were subjected to non-denaturing 6% polyacrylamide gel electrophoresis in 0.25 × TBE at 4 °C for 75 min (at 10 mA constant). Gels were dried and analyzed by Phosphoimager (Typhoon FLA 7000).

3. Results

3.1. Transcriptional activation of human cytomegalovirus immediate early promoter with dexamethasone (DEX)

To examine whether the increase of MIE protein synthesis facilitated by DEX is dependent on transcriptional activation of the MIE gene, we first performed quantitative RT-PCR to quantitatively determine the viral MIE gene mRNA level in HCMV-infected cells (Fig. 1A). In cells pre-treated with 1 μM DEX for 24 h prior to infection, we found that the mRNA level of the MIE gene is approximately 2 times higher than that of infected cells without DEX, suggesting that the MIE gene mRNA level is increased by DEX treatment.

Next, we constructed a *luciferase* reporter plasmid by cloning the HCMV genomic DNA fragment between nucleotide positions of 1994 bp upstream and 121 bp downstream of the transcription start site including promoter, enhancer, and exon 1 (pMIEP-Luc-1994/+121) of the MIE gene (Fig. 1B). The MIE gene exon 1 corresponding to 5′ UTR is common among alternative MIE transcripts [14]. To address whether the exon 1 of the MIE gene is involved in DEX-mediated increase of the MIE gene expression, we also constructed *a luciferase* reporter plasmid lacking almost all region of exon 1 (pMIEP-Luc-1994/+10) (Fig. 1C). In the presence of every concentration of DEX, the relative luciferase unit was increased approximately 1.5–2 times higher than that of non-treated cells transfected with pMIEP-Luc-1994/+10 similarly to the observation when pMIEP-Luc-1994/+121 was used (Fig. 1D). These results suggest that the upstream region of the MIE gene transcription start site, but not the MIE gene exon 1 region, is involved in the DEX-mediated transcriptional activation.

To determine which region within the 2 kbp-long MIE regulatory region is involved in DEX-mediated transcriptional activation,