Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 368 (2008) 977–982
BBRC
www.elsevier.com/locate/ybbrc

PTH regulation of the human cytomegalovirus immediate-early gene promoter

Johann Herberth [a,*], Alexander P. Alimov [b], John P. Williams [a,1], Hartmut H. Malluche [a], Nicholas J. Koszewski [a]

[a] University of Kentucky Medical Center, Division of Nephrology, Bone and Mineral Metabolism, Room MN562, 800 Rose Street, Lexington, KY 40536-0298, USA
[b] University of Cincinnati, Department of Surgery, 234 Goodman Street, Cincinnati, OH 45219, USA

Received 5 February 2008
Available online 13 February 2008

Abstract

Secondary hyperparathyroidism and human cytomegalovirus (hCMV) seropositivity are highly prevalent in patients undergoing renal transplantation, and both are linked to the development of chronic allograft nephropathy (CAN). We investigated the hypothesis that parathyroid hormone (PTH) 1–84 regulates hCMV immediate-early gene (IEG) promoter activation in proximal renal tubular cells. PTH 1–84 enhanced hCMV IEG promoter (−548 to +92) activity in opossum kidney cells. Deletion analysis from the 5′ end of the promoter localized the PTH 1–84 associated activity to the DNA sequence between −123 and −45. Mutation of an imperfect ATF/AP-1 DNA element within this region abrogated the PTH 1–84 effect and also strongly attenuated basal gene expression. Mobility shift analyses using this DNA element revealed that a member of the ATF-1 family was in the binding complex. In summary, we present evidence for a novel pathogenic role of PTH 1–84 in promoting hCMV immediate-early gene transcription.

Keywords: Parathyroid hormone; Human cytomegalovirus; Immediate-early gene; Transcription; CREB; ATF-1; DNA; Kidney; Chronic allograft nephropathy; Tubular cells

Human cytomegalovirus (hCMV) is a herpes virus affecting 70–100% of the population worldwide. In the United States, the prevalence of serologic evidence of hCMV infection in individuals older than 6 years of age is estimated at 58.9% with a gradual increase in age up to 90.8% in octogenarians [1]. For many years, hCMV was regarded as the responsible organism only for rare neonatal diseases but the emergence of diseases leading to compromised cellular immunity established hCMV as an important determinant of morbidity and mortality in patients with human immunodeficiency virus (HIV) infection or on immunosuppressive therapy for organ transplantation [2].

In patients undergoing renal transplantation, the prevalence of hCMV seropositivity exceeds 60% [3], and the hCMV infection rate 44% [4]. Despite antiviral prophylaxis, 5–18% of organ transplant recipients develop hCMV disease [5]. Both, the transplanted and the native kidneys have been described as frequent sites for hCMV latency [6], and hCMV infection has been recognized as an independent risk factor for chronic renal graft rejection [7]. Solà and colleagues reported a direct proportionality between hCMV viral load and the risk of developing histological features of chronic allograft nephropathy (CAN) [8]. Immunohistochemistry and in situ DNA hybridization demonstrated expression of the hCMV genome in tubular cells of renal allografts even during latency without detectable hCMV antigenemia [9].

Hyperparathyroidism is present in 63% of patients prior to receiving a renal allograft [10], persists in up to 50% of

* Corresponding author. Fax: +1 859 323 0232.
E-mail address: jherb2@uky.edu (J. Herberth).
[1] Present address: National Institute of Aging, Biology of Aging, 7201 Wisconsin Avenue, Bethesda, MD 20814, USA.

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.02.019

patients one year post transplantation [11], and has been associated with inferior renal graft function [12] and the development of histological features of CAN [13]. Interestingly, the promoter region of the hCMV early gene exhibits multiple potential sequences for PTH regulation including elements recognized by the cyclic AMP response element-binding protein/activating transcription factors (CREB/ATF), the CAAT/enhancer-binding proteins (C/EBP) and nuclear factor kappa β (NF-κβ [14,15]. Using opossum kidney (OK) cells as a model for proximal renal tubular cells, we investigated the hypothesis that PTH 1–84 regulates hCMV early gene promoter activity.

Materials and methods

OK cell line was purchased from the American Type Culture Collection (Manassas, VA). Lipofectamine and Plus reagent were purchased from Invitrogen Life Technologies (Carlsbad, CA). Luciferin assay reagent and cell lysis buffer were purchased from Promega Corp. (Madison, WI). Protease inhibitor cocktail was purchased from Roche Molecular Biochemicals, (Indianapolis, IN). Oligonucleotides were synthesized by Integrated DNA Technologies (Coralville, IA). Enzymes were purchased from New England Biolabs (Ipswich, MA) unless otherwise indicated. The hCMV immediate-early β-galactosidase (hCMV-βgal) reporter vector was a generous gift from Dr. D. Noonan, University of Kentucky, Lexington, KY. The expression vector for the human PTH type 1 receptor (hPTH-1R) was a generous gift from Dr. E. Schipani, Massachusetts General Hospital-Harvard Medical School, Boston, MA. PTH 1–84 was synthesized by New England Peptide (Gardner, MA). Forskolin and phorbol 12-myristate 13-acetate (PMA) were purchased from Sigma–Aldrich Co. (St. Louis, MO). Anti-C/EBP (SC-746) and CREB (SC-186) antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

Preparation of promoter reporters. A 1.3 kb human 1α-hydroxylase (h1α-Hyd) promoter fragment was PCR amplified from human genomic DNA using the following primers: 5′-ATGGATCCGAGGGAGTA AGGAGCAGAGAGGTAAACTG and 5′-ATCTCGAGCGAAAGA AAGCGCTTCTCCTGAGC. The PCR product was digested with the combination of BamHI/XhoI, gel purified and ligated into the BglII/XhoI sites of a modified pGL3p vector (pGL3linker) whereby the SV40 promoter was previously removed by digestion with SacI/HindIII and replaced with a cloning insert: SacI–BglII–SmaI–XhoI–HindIII.

Deletions of the hCMV promoter were created by PCR using the following primers: −428, 5′-CGGAATTCCCAATAGGGACTTTCCA TTGACGTCA; −301, 5′-CGGAATTCCCTGGCATTATGCCCAGTA CATGACCT; −187, 5′-CGGAATTCGTGGATAGCGGTTTGACTCA CGGGG; −123, 5′-CGGAATTCCGGGAGTTTGTTTTGGCACCAA; −45, 5′-CGGAATTCGGTAGGCGTGTACGGTGGGAGGTCT.

These were individually paired with the 3′ primer hCMVrev, 5′-AGTTCCTCGAGTACCGGATCCTCTAGAG and PCR amplified. Products were digested with EcoRI/XhoI, gel purified and ligated into the same sites of the parent vector following excision of the wild-type hCMV promoter with the same enzymes.

Mutations were incorporated into hCMV/−123 promoter constructs by two-step PCR as previously described [16]. Primers for selective inactivation of the transcription factor binding sites (mutations underlined): NF-κβ, 5′-CAACCCCTCTTTAAAAAATGTCGTAACAACTCCGCC; C/EBP, 5′-TTCCAAACCGCTACAACAACTCCGCCCCATTG; ATF, 5′-CCGCCCCTGAAATTTAAATGGGCGGTAGGCGTGTACG.

A similar strategy was utilized to generate hCMV/−428 mutant ATF construct with the exception being the use of the hCMV/−428 primer. All mutant promoter constructs were sequenced manually to confirm their DNA identity.

The hCMV-pGL3 reporter was prepared as follows; the pGL3linker reporter was digested with SmaI/XhoI, treated with phosphatase and gel purified. The hCMV-βgal vector was initially digested with EcoRI, the ends blunted with T4 DNA polymerase and subsequently digested with XhoI. The excised hCMV promoter fragment was gel purified and ligated with the digested pGL3linker reporter to yield the hCMV-pGL3 reporter.

Transient transfection studies. OK cells were transfected in 24-well plates with 500 ng DNA/well as previously described [17], followed by supplementation to 1% serum for 18 h. Cells were then treated with or without various compounds for 24 h as indicated in Figure legends. Luciferase and β-galactosidase activities from individual wells were determined, average mean values and standard errors calculated, and are reflective of 2–3 independent experiments. Best-fit curves, half-maximal values and statistical comparisons were obtained with PSI-Plot software (Pearl River, NY).

Nuclear extracts and mobility shift assays. Mobility shift assays and OK cell nuclear extracts were prepared as described previously [16], with the exception that extract suspensions were clarified at ca. 15,000*g*.

Results

When the β-galactosidase reporter vector under the control of the hCMV major immediate-early promoter was transfected into OK cells, a strong increase in activity was seen in response to PTH 1–84 (Fig. 1A). This was observed with the native cells, as well as those transfected with increasing amounts of hPTH-1R. The h1α-Hyd promoter activity was relatively unchanged in response to PTH 1–84 (Fig. 1B), and was nominally used to monitor transfection efficiency. This is consistent with previous work indicating OK cells lacked endogenous expression of the 1α-hydroxylase gene [18].

Dose–response was then assessed by treating OK cells with varying concentrations of PTH 1–84 in the presence

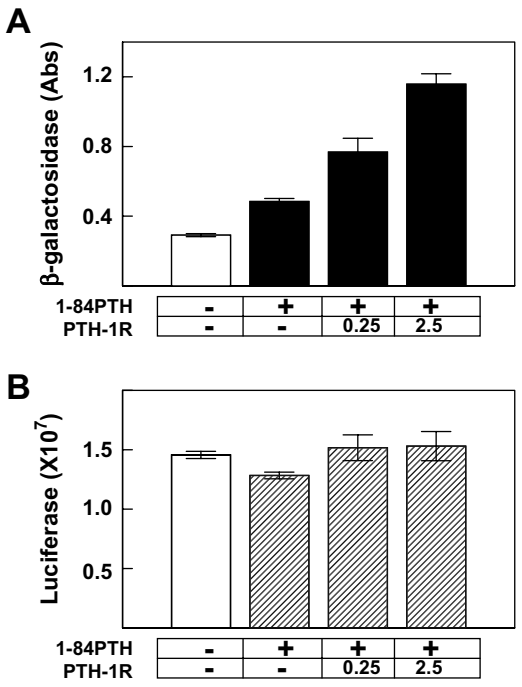

Fig. 1. Cells were co-transfected with hCMV-βgal (10 ng), and h1α-Hyd luciferase (10 ng) reporter vectors in the absence or presence of an expression vector for the hPTH-1R (ng/well). Cells were treated with 50 nM PTH 1–84 and analyzed for β-galactosidase (A) and luciferase (B) activities.