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HPV E7 viral oncoprotein disrupts transcriptional regulation of *L1Md* retrotransposon

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ABSTRACT

Murine L1Md-A5 retrotransposon is a redox-inducible element regulated by Nrf-2/JunD and E2F/Rb-binding sites within its promoter (5′-UTR). Because the human papillomavirus (HPV) oncoprotein E7 interacts with retinoblastoma (pRb) and members of the AP1 family, studies were conducted to examine functional interactions between HPV E7, pRb, and histone deacetylase 2 (HDAC2) in the regulation of L1Md-A5. Using a transient heterologous transcription system we found that HPV E7 alone, or in combination with HDAC2, disrupted pRb-mediated L1MdA-5 transactivation. HPV E7 also ablated the transcriptional response of L1Md-A5 to genotoxic stress, but did not interfere with basal activity. We conclude that HPV E7 associates with proteins involved in the assembly of macromolecular complexes that regulate antioxidant and E2F/Rb sites within L1MdA-5 to regulate biological activity

1. Introduction

Long interspersed nuclear elements (LINE-1 or L1) are genetic mobile retrotransposons that insert into the genome via a "copy-and-paste" mechanism using a self-encoded reverse transcriptase and RNA intermediates [1]. The full-length consensus human L1 (L1h) is a 6–7 kb DNA sequence [2,3] that consists of a 903 bp 5′-untranslated region (5′-UTR) [4] containing a bidirectional RNA polymerase II internal promoter, followed by two open reading frames (ORF1 and ORF2), a 3′-UTR containing an AATAAA polyadenylation signal and a poly A tail [1]. Translation of the L1 mRNA leads to expression of ORF1p, a 40 kDa protein [4], with nucleic acid and protein-protein-binding capabilities [5], and ORF2p, a 150 kDa protein [6] with endonuclease [7] and reverse transcriptase activities [8]. Murine L1 (L1Md) retroelements are similar in function to L1h. In contrast to humans where only one active L1 family exists [9], structural differences within the 5′-UTR region of murine L1 give rise to several lineages, namely, F, G, Tf, Gf and A [10]. The A-type 5′-UTR is made of tandemly arranged 208 bp-long monomeric DNA repeats that vary in number [11]. The promoter strength is directly related to the monomer number within the 5′-UTR [11,12].

Our laboratory has previously identified a novel retrotransposon, L1MdA5, as a target retroelement in the genotoxic stress response to benzo-a-pyrene (BaP), an environmental carcinogen [12]. Subsequent studies identified Nrf-2/JunD and E2F/Rb binding sites within the promoter that participate in transcriptional regulation of the L1 promoter [12,13], and established a mathematical model of retrotransposon reactivation by BaP in heterologous systems [14]. Given that many of the proteins involved in transcriptional control of L1 interact with human papillomavirus (HPV) E6 and HPV E7 oncoproteins [15–17], we hypothesized that a functional link exists between stress and viral protein-regulated gene expression. Here we show that transient heterologous overexpression of Rb in human cervical cancer cells (HeLa cells) as well as challenge with BaP transactivate the L1 promoter, and that forced overexpression of HPV E7 decreased the transcriptional response of the luciferase reporter gene to both Rb and carcinogen challenge.

2. Materials and methods

2.1. Cell culture

Human cervical cancer-derived HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL, Carlsbad,

Abbreviations: 5′-UTR, 5′-untranslated region; AdE1A, adenovirus oncoprotein early region 1A; BaP, benzo(a)pyrene; CNC-bZIP, cap n′ collar-basic leucine zipper protein family; DNMT, DNA methyltransferase; DMSO, dimethyl sulfoxide; EpRE-like, electrophile-like response element; HDAC, histone deacetylase; HMT, histone methyltransferase; HPV, human papillomavirus; LINE1 or L1, long interspersed nuclear element; MEF, mouse embryo fibroblast; ORF, open reading frame; Rb, retinoblastoma

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CA) supplemented with 10% FBS and 1% penicillin-streptomycin antibiotics (Cellgro, Manassas, VA), and kept at 37 °C and 5% CO_2.

2.2. Transient transcription assays

Hela Cells were plated at a density of 1.5×10^4 cells/well in 24-well plates. Transient transfections with 500 ng of pBASIC-luciferase or pL1Md-A5 Wild Type-luciferase reporter gene constructs [12] with or without 100 ng of HPV E7, and/or Rb or HDAC2 overexpression vectors, were performed using the Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA). Transient transfections were performed according to manufacturer's specifications. Briefly, cells were plated in 24-well plates the day before transfection and maintained in growth medium without antibiotics. The growth medium was replaced with serum-free Opti-MEM medium the following day. DNA-Lipofectamine complexes were added to the cells and transfections allowed to continue for 6 h. Next, the transfection medium was aspirated and 0.5 ml of fresh medium without antibiotics added to each well. Cells were allowed to recover for 18 h before initiation of chemical treatments [18]. To correct for randomness during transfection, 10 ng of the Renilla luciferase reporter gene (pRL) was cotransfected in each assay. The total DNA load per transfection was kept constant at 800 ng with pBlueScript. Cells were lysed 36 h after transfection and the luciferase activity measured using a Dual-Luciferase Reporter Assay System protocol (Promega, Madison, WI). Measurements were performed on a 20/20 Luminometer (Turner BioSystems, Sunnyvale, CA). Corrected Arbitrary Luciferase Units (ALU) were calculated as the ratio of activity of the pBASIC or the pL1Md-A5 luciferase reporter gene and the pRL activity. Each assay was performed in triplicate and at least two independent assays were performed for each experiment. The data shown are from one of the representative experiments and the error bars represent the standard deviation from triplicate assays.

2.3. Chemical treatments

Twenty-four hours after transfection, fresh medium containing 0.06% (V/V) dimethyl sulfoxide (DMSO) vehicle or 3 μM BaP was added to the cells for 16 h, as previously reported [18]. At the concentrations used in our experiments DMSO did not activate cellular redox signaling. Cells were subsequently processed for luciferase activity quantification as described above.

2.4. Statistical analyses

Analyses were done either by student's t test or ANOVA ($P < 0.05$) followed by post-hoc tests.

3. Results

3.1. Rb protein and HPV E7 oncoprotein exert opposite effects on the mouse L1MdA5 retroelement promoter

We previously identified L1MdA5 as a redox-inducible element [12] containing two functional electrophile response elements (EpRE)-like elements (Fig. 1A). We also have reported that E2F/pRb complexes bind to, and regulate, mouse L1 elements through mechanisms that involve HDAC proteins and epigenetic repression [13]. Since HPV E6 and HPV E7 oncoproteins interact with pRb and inactivate its cell cycle-related repressor function, we hypothesized that overexpression of HPV E7 oncoprotein alters L1 promoter activity. Thus, the effects of forced Rb and HPV E7 overexpression on L1MdA5 5′-UTR in vitro were investigated [12]. Transient transfections of HeLa cells with 500 ng of pBASIC-luciferase control or pL1Md-A5 Wild Type-luciferase reporter gene constructs [12,18] (Fig. 1B), in the presence or absence of 100 ng of Rb or HPV E7 expression vectors, were performed. To correct for transfection artifacts, 30 ng of the Renilla luciferase reporter gene (pRL) were cotransfected in each assay. Thirty six hours after transfection, cells were lysed and luciferase activity measured. Transfection of the pBASIC vector alone, or in combination with Rb, HPV E7, or both, yielded low levels of corrected ALU, showing that the weak basal promoter activity of the parent vector was not affected by these proteins (Fig. 2A). In contrast, placement of the pL1Md-A5 5′-UTR sequence upstream of the luciferase reporter gene showed strong promoter activity, as evidenced by increased corrected ALU for the L1MdA5 promoter alone (0.012 vs. 1.4 ALU for pBASIC versus pL1Md-A5, respectively) (Fig. 2B). Expression of Rb increased pL1Md-A5 activity over the pBASIC vector (Fig. 2A), and over wild type controls (Fig. 2B), and this response was inhibited by HPV E7 viral oncoprotein (Fig. 2B). HPV E7 expression vector alone did not alter the corrected luciferase reporter activity of pL1Md-A5 (Fig. 2B). To further investigate the role of Rb in transactivation of L1MdA5, forced overexpression of HDAC2, a corepressor protein was examined [13,19]. HeLa cells were cotransfected with pBASIC or pL1Md-A5 luciferase reporter vectors in the presence of 100 ng of Rb, HDAC2 or HPV E7 expression vectors, or their combination as indicated (Fig. 3A). HDAC2 alone did not change corrected luciferase reporter activity (Fig. 3B). When coexpressed with Rb, HDAC2 did not disrupt Rb-mediated transactivation of the L1Md promoter in vitro (Fig. 3B). In contrast, coexpression of Rb, HDAC2 and HPV E7 led to strong inhibition of L1MdA5 transactivation, indicating that HPV E7 in association with HDAC2 blocks pRb effects. In all, these data suggest that inhibitory effects of HPV E7 are mediated through functional interactions with both pRb and HDAC2.

3.2. HPV E7 ablates the response of the L1MdA5 reporter to genotoxic stress

Since pRb is a frequent target of viral oncoproteins including adenovirus oncoprotein early region 1A (AdE1A) and HPV E7 [20,21], and E2F/pRb complexes interact with human and mice L1 5′-UTR regions [13], we hypothesized that HPV E7 overexpression alters the regulatory transactivation role for pRb on pL1Md-A5 under conditions of genotoxic stress. To this aim, we transiently transfected HeLa cells with pBASIC or pL1Md-A5 luciferase reporter vectors in the presence or absence of HPV E7 expression vector, followed by 3 μM BaP or control DMSO [18] (Fig. 4A and B, respectively). Experiments conducted with pBASIC luciferase reporter alone showed low levels of corrected luciferase ALU for both DMSO and BaP treated cells (Fig. 4A). When cells were cotransfected with pBASIC and HPV E7 expression vector a similar result was obtained indicating that DMSO, BaP or HPV E7 did not influence the transcriptional activity of the control reporter vector. When cells were transiently transfected with pL1Md-A5 vector alone followed by exposure to 3 μM BaP, the corrected reporter gene ALU showed a robust response compared to control DMSO (Fig. 4B). Importantly, BaP treatment elicited significant changes in the reactivation of the pL1Md-A5 reporter under all experimental conditions tested. DMSO alone increased reporter activity, a finding consistent with the redox-modulating effects of this solvent [12]. Forced overexpression of HPV E7 oncoprotein ablated the response of pL1Md-A5 reporter vector to genotoxic stress, but did not modify the response of the control pL1Md-A5 to DMSO (Fig. 4B). These data indicate that viral oncoproteins interfere with the cellular machinery responsible for L1 reactivation under conditions of genotoxic stress.

4. Discussion

HPV E6 and HPV E7 proteins target a diverse variety of host cellular proteins involved in cytoplasmic and nuclear protein complex