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ScienceDirect
Virus Research 131 (2008) 1–7
Virus Research
www.elsevier.com/locate/virusres

The interaction between KSHV RTA and cellular RBP-Jκ and their subsequent DNA binding are not sufficient for activation of RBP-Jκ

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Received 20 April 2007; received in revised form 30 July 2007; accepted 30 July 2007
Available online 11 September 2007

Abstract

Kaposi's sarcoma-associated herpesvirus (KSHV) replication and transcription activator (RTA) is necessary and sufficient for the switch from KSHV latency to lytic replication. RTA activates promoters by several mechanisms. RTA can bind to sequences in viral promoters and activate transcription. In addition, RTA interacts with the cellular recombination signal sequence-binding protein-J kappa (RBP-Jκ), a transcriptional repressor, converts the repressor into an activator and activates viral promoters via RBP-Jκ. Because RBP-Jκ is required for RTA to activate lytic replication, it is important to understand how RTA cooperates with RBP-Jκ protein to activate KSHV lytic replication program. Previously, we identified an RTA mutant, RTA-K152E, which has a defect in its direct DNA-binding activity. In this report, the effect of the mutant RTA on KSHV activation via RBP-Jκ protein is examined. We demonstrate that RTA-K152E interacts with RBP-Jκ physically and the mutant RTA and RBP-Jκ complex binds to target DNA properly in vivo and in vitro. However, the complex of RTA-K152E and RBP-Jκ does not activate transcription. Furthermore, the RTA mutant (RTA-K12E) inhibits cellular Notch-mediated RBP-Jκ activation. These data collectively suggest that the complex between KSHV RTA and cellular RBP-Jκ and the subsequent DNA binding by the complex are not sufficient for the activation of RBP-Jκ protein. Other factor(s) whether additional cofactor(s) in the complex or the intrinsic conformation of RTA, are predicted to be required for the activation of RBP-Jκ protein by RTA.

Keywords: KSHV; RTA; RBP-Jκ; Lytic replication; HHV8; PEL

1. Introduction

Kaposi's sarcoma (KS)-associated herpesvirus (KSHV), also known as human herpesvirus 8 (HHV-8), is a gamma herpesvirus. It is believed to be the etiological agent of KS (Chang et al., 1994; Chang and Moore, 1996; Moore and Chang, 2001). KSHV is also implicated in the pathogenesis of AIDS-associated primary effusion lymphoma (PEL, also called body cavity-based lymphoma (BCBL)) and a lymphoproliferative disorder known as multicentric Castleman's disease (Dourmishev et al., 2003; Moore and Chang, 2001; West and Wood, 2003).

As other herpesviruses, KSHV goes through both latency and lytic replication cycles. The expression of the KSHV replication and transcription activator (RTA) is necessary and sufficient for the switch from latency to lytic replication (Dourmishev et al., 2003; West and Wood, 2003). RTA is an immediate early gene (Sarid et al., 1998; Sun et al., 1999; Zhu et al., 1999) and a sequence-specific DNA-binding protein. A number of RTA-responsive elements (RRE) were identified in the transcriptional regulatory regions of different subsequently expressed viral genes (Gradoville et al., 2000; Lukac et al., 1999; Sakakibara et al., 2001; Saveliev et al., 2002; Sun et al., 1998, 1999; Zhu et al., 1999).

In addition to direct DNA binding, the ability of RTA to interact with other factors appears to be necessary for activating its transcriptional potential (Gwack et al., 2001, 2002; Liao et al., 2003; Wang et al., 2001). One of the interacting factors is the cellular recombination signal sequence-binding protein-J kappa (RBP-Jκ) (also known as CBF-1 and CSL) (Carroll et al.,

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doi:10.1016/j.virusres.2007.07.019

2006; Liang et al., 2002; Liang and Ganem, 2003). RBP-Jκ is a sequence-specific DNA-binding protein and a transcriptional repressor that is involved in the Notch signaling pathway.

The Notch signaling pathway regulates cell fate in a variety of organisms (Bray, 2006; He and Pear, 2003). Interaction of Notch receptors (Notch) with their ligands leads to cleavage of the Notch intracellular domain (NICD), which leads to nuclear localization of NICD. In the nucleus, NICD associates with RBP-Jκ and converts RBP-Jκ from a transcriptional repressor to an activator. Furthermore, the NICD-RBP-Jκ complex activates expression of specific target genes (Bray, 2006; He and Pear, 2003).

KSHV RTA can directly bind to RBP-Jκ and the RTA–RBP-Jκ complex binds to DNA and activates transcription of KSHV promoters (Carroll et al., 2006; Liang et al., 2002; Liang and Ganem, 2003). The KSHV genome contains numerous RBP-Jκ elements suggesting that RBP-Jκ plays an important role in viral transcription. The interaction between RTA and RBP-Jκ is essential for the switch from viral latency to lytic replication in rodent cells (Liang and Ganem, 2003). However, it is not clear if interaction between RTA and RBP-Jκ is the only event that is necessary for activating KSHV lytic gene expression.

Previously, we have identified a novel function of RTA, i.e., RTA binds to interferon-stimulated response elements (ISRE) and activate certain cellular interferon-stimulated genes (ISG) (Zhang et al., 2005). In addition, a region in RTA DNA-binding domain has been identified with certain sequence similarity to the DNA-binding domains of interferon regulatory factor (IRF) family. Mutation in one conserved amino acid within this region (RTA-K152E) reduces the ability of RTA to bind to ISRE as well as other RREs. Furthermore, the RTA-K152E fails to activate RTA-responsive promoters and to induce viral lytic gene expression (Zhang et al., 2005).

In this report, we have further characterized the RTA-K152E mutant in terms of activating the cellular RBP-Jκ protein. The mutant RTA is able to interact with RBP-Jκ physically and the RTA-K152E-RBP-Jκ complex is able to bind to DNA in vitro and in vivo. However, the mutant failed to activate RBP-Jκ protein. Thus, the complex between KSHV RTA and cellular RBP-Jκ and their subsequent DNA binding are not sufficient for the activation of RBP-Jκ protein.

2. Materials and methods

2.1. Plasmids and antibodies

Expression plasmids of FLAG-tagged RTA and mutant RTA-K152E were previously described (Zhang et al., 2005). The HA-tagged RBP-Jκ expression plasmid was a gift from Dr. Paul Ling (Baylor College of Medicine). FLAG-tagged RBP-Jκ expression plasmid and the reporter construct containing 3× RBP-Jκ binding site were gifts from Drs. Don Ganem and Yuying Liang (Liang et al., 2002). The constitutively active Notch expression plasmid, pcDNA-3-mNotch-ΔE, was a gift from Dr. Franz Oswald (Oswald et al., 2002). CMV-β-gal and CMV-GFP expression plasmids were from Clontech. Peptide RTA antibody was described (Xu et al., 2007). Tubulin and FLAG-antibodies were purchased from Sigma. HA and RBP-Jκ antibodies were purchased from Santa Cruz.

2.2. Western blot analysis, cell culture, transient transfection and reporter assays

Standard western blot analysis was performed as previously described (Zhang and Pagano, 1997, 1999, 2000, 2001). 293-Bac (a gift from Dr. S.J. Gao) is a human 293 cells derived cell line and containing KSHV genome (Zhou et al., 2002). 293-Bac36 cells were maintained in DMEM plus 10% fetal bovine serum (FBS) plus 0.5 mg/ml hygromycin. 293T cells are a human fibroblast line and were maintained in DMEM plus 10% FBS. Effectene (Qiagen) was used for the transfection of these cells. The luciferase assays were performed using a kit from Promega according to manufacturer's recommendation.

2.3. Preparation of KSHV stocks and detection of virion DNA

293-Bac36 cells were transfected with the designated expression plasmids and culture supernatants were harvested 5 days later. Virions were pelleted by centrifugation at 100,000 × *g* for 1 h. The pellets were subsequently suspended in 1× PBS buffer (1:100 of the volume of the original supernatants). The concentrated viruses were then treated by DNase I at 37 °C for 1 h. DNA was extracted and PCR analyses were carried out with primers targeted K14/vGCR region (BC1 KSHV genome coordinates: 127,649–127,883). The conditions of the PCR assay were established empirically to ensure a linear amplification of template DNA within the amplification conditions.

2.4. Chromatin immunoprecipitation (ChIP)

293-Bac36 cells were transfected with various plasmids. One day later, ChIP assays were performed using the chromatin immunoprecipitation assay kits according to the manufacturer's recommendation (Upstate, Inc.). Anti-FLAG and normal rabbit serum (NRS) was used as designated. The DNA exacted from immunoprecipitates was used as template for PCR analyses with various primers that amplify MTA- and PAN-specific products (Wang et al., 2003a,b). PCR products were resolved in 8% polyacrylamide gel.

2.5. Co-immunoprecipitation (Co-IP)

293T cells grown in 10 cm plates were transfected with the designated plasmids. Cell extracts were prepared using RIPA buffer (1× PBS, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with a protease inhibitor cocktail (Roche) were incubated with either mouse anti-FLAG monoclonal antibody M2 (Sigma) or anti-HA antibody (Santa Cruz) for 1 h on ice. Protein G-sepharose (Pharmacia) was added and the incubation continued at 4 °C overnight with gentle rotation. Beads were washed three times with 1× PBS buffer and boiled in SDS loading buffer and subsequent western blot were essentially the same as described.