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LETTER TO THE EDITOR

A silence element involved in Kaposi′s sarcoma-associated herpesvirus ORF11 gene expression

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Kaposi′s sarcoma-associated herpesvirus (KSHV also known as Human herpesvirus 8) is the etiological agent of Kaposi′s sarcoma (KS) (*1, 2, 12, 15*), a disease of endothelial origin (*14*). KSHV infection has also been implicated in the etiology of two B-cell lymphoproliferative diseases, e.g. primary effusion lymphoma and multicentric Castleman's disease (*3, 4, 18*). The KSHV ORF11 is a lytic viral gene with delayed-early expression kinetics (*7*). Little is known about ORF11 protein function in KS or primary effusion lymphoma tumors. The discovery of a dUTPase like domain in ORF11 coding sequence suggests that ORF11 protein may function as a dUTPase (*8*). It is still controversial, whether ORF11 functions like the dUTPase, since the functional dUTPase activity has not been formally demonstrated (*16*). The association of ORF11 with tegument protein ORF45 suggests that ORF11 may function in KSHV virion morphology, latency establishment, or host interaction (*22*).

In our previous work, we showed that deletion of vIL-6 led to the significantly increased expression of ORF11 in both un-induced and induced BJAB (Epstein-Barr virus-negative, Burkitt-like lymphoma) cells harboring vIL-6 deletion mutant (*5, 6*) indicating the interference of ORF11 promoter activity by the deletion of vIL-6 gene. Viral promoter activity may be controlled by the tightly regulated molecular switch formed by a balanced array of positive and negative factors (*13*). Deregulated viral or cellular factors resulted from the deletion of vIL-6, such as vIL-6 or cellular factors regulated by the vIL-6/gp130 signaling pathways, may interfere with the ORF11 promoter activity in such a way that the ORF11 gene expression is no longer regulated by the KSHV lytic program. Interestingly, no RTA-responsive element has been identified in the ORF11 promoter suggesting an indirect regulation of ORF11 by KSHV RTA (*7*). Similarly, computational analysis of potential transcription factor binding sites in the ORF11 promoter did not reveal any binding sites for the transcription factors regulated by gp130 signaling pathways (*7*). Above data suggested that the ORF11 expression was independent of the vIL-6 protein. However, we cannot rule out the possibility that other viral factors act synergically with vIL-6. In the absence of such viral proteins, vIL-6 may not be able to regulate ORF11 expression. In addition, it is possible that some cell type specific factors, such as those of hematopoietic origin are involved in the regulation of ORF11 expression. Such cell type specific factors may not exist or be functional in the HEK293 cells (epithelial cell line).

Based on the fact that vIL-6 protein did not interfere with ORF11 promoter activity, we hypothesize that a putative inhibitory *cis*-regulatory element may be deleted in the KSHV genomic region (17,090–17,568 nts, BC-1 position). Negative regulatory elements had been discovered in human herpesviruses like human cytomegalovirus (*19*) and KSHV (*21*), papillomaviruses like human papillomavirus type 8 (*17*) and bovine papillomavirus type 4 (*20*), and other human viruses like hepatitis B virus (*10*). Identification and characterization

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Abbreviations: eGFP = enhanced green fluorescence protein; KSHV = Kaposi′s sarcoma-associated herpesvirus; KS = Kaposi′s sarcoma; RTA = replication and transcription activator; vIL-6 = viral IL-6

such a silencer element helps to deepen our knowledge of KSHV ORF11 gene expression and regulation.

To determine, if such a negative regulatory element was deleted in the KSHV genomic region, various lengths of putative negative elements downstream of the ORF11 polyadenylation signal were PCR-amplified using the following primers (restriction enzyme sequences underlined; shared forward primer). R1-F: 5′-AGTGTCGACACTA GTATTGTGTTCCCA-3′; R1-R: 5′-AGTGTCGACGAA TTCGAGGTGTTGTT-3′; R2-R: 5′-AGTGTCGACGAAT TCAAGAGCGACGTGACATAA-3′. Resulting DNA fragments were inserted into the SalI site of the reporter plasmid pGL3-209 in both sense and anti-sense orientation. A 480 bp GFP DNA fragment was PCR-amplified using the following primers: GFP-F: 5′-AGTGTCGACACTAGTG TGACCACCCTGACCTACGGC-3′; GFP-R: 5′-AGTGT CGACGAATTCCAGGACCATGTGATCGC-3′. Resulting DNA fragments were inserted into the SalI site of reporter plasmid pGL3-209 in the sense orientation. The resulting plasmid pGL3-209-GFP was used as a negative control in dual luciferase reporter assays. Other reporter plasmids used in this study were described (*7*). HEK293 cells were transfected in a six-well plate using the Mirus TransIT-293 reagent (Mirus) with 2 μg of the reporter plasmid containing various lengths of putative negative elements as well as 2 ng of control Renilla luciferase expression vector pRL-SV40 (Promega) for a transfection efficiency normalization. Luciferase assay was performed on the cell lysates 30 hrs after transfection with the dual reporter luciferase kit (Promega) following manufacturer's instructions. Firefly luciferase expression in HEK293 cells was normalized to that of Renilla luciferase activity.

As shown in the Fig. 1a, the presence of R1 element (17,090–17,568 nts, BC-1 position) significantly decreased the ORF11 promoter activity by 66%, while R2 element (17,090–17,261 nts) and eGFP element only slightly decreased ORF11 promoter activity by 9.8% and 6.4%, respectively. The presence of R1 element in antisense orientation still decreased the ORF11 promoter activity by 41%. Above data indicated that a silencer element existed in the genomic

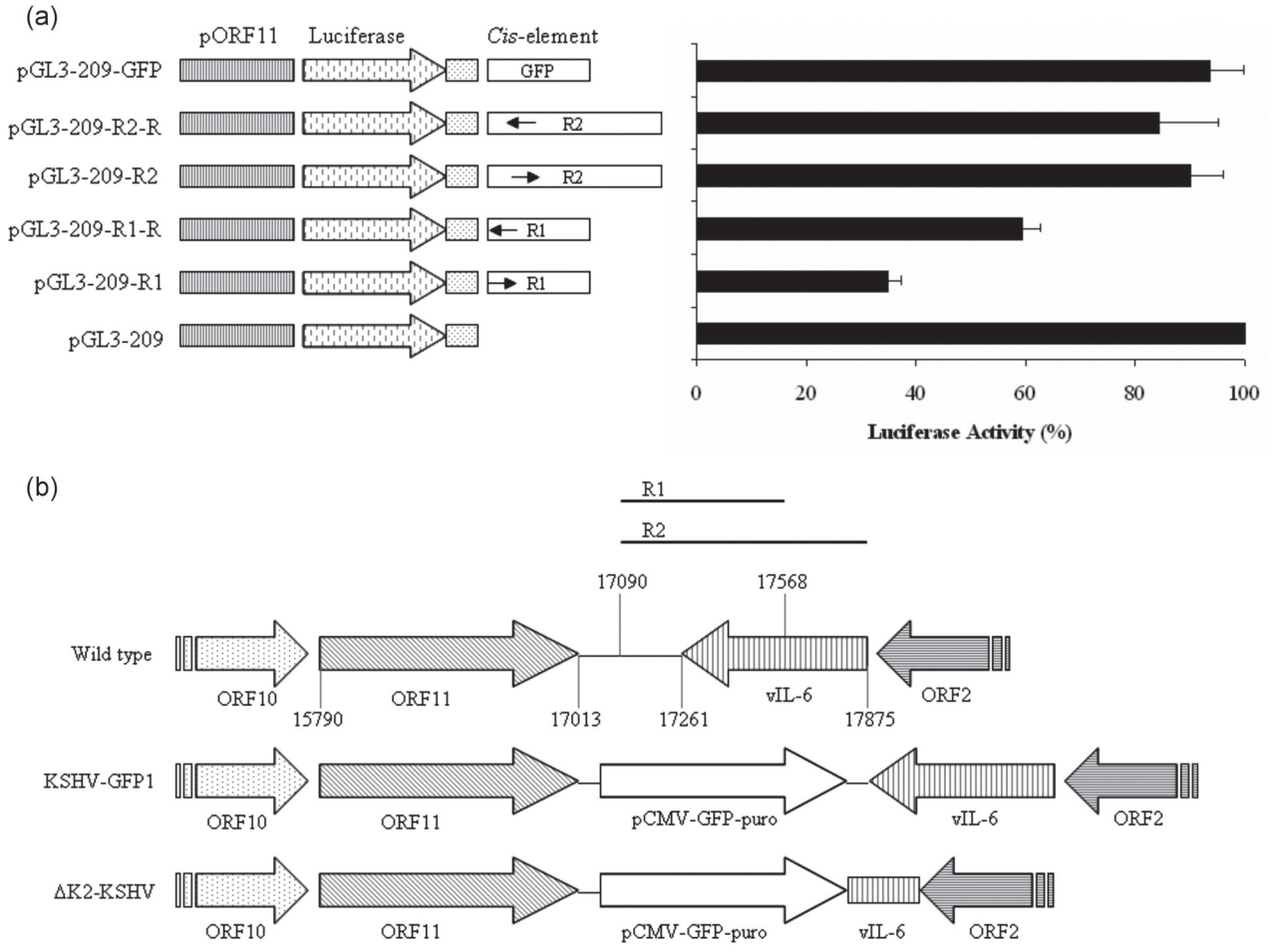

Fig. 1

region of 17,261–17,568 nts. Interestingly, the suppression of ORF11 promoter activity by this silencer element was position-independent. ORF11 promoter activity was not interfered by the insertion of a 2.2kb eGFP/puromycin selective marker sequence between the ORF11 promoter and silencer element (Fig. 1b). Such position-independent silencing effects were previously reported in the regulation of human IL-4 and Vimentin expression (*9, 11*). Those silencer elements discovered in the IL-4 and Vimentin promoters interfered with basal promoter activities through the suppression of enhancer elements (*9, 11*). However, no enhancer element has been identified in the ORF11 promoter so far (*7*). This may indicate a novel mechanism of gene regulation utilized by the KSHV to precisely control the ORF11 gene expression. The dramatic up-regulation of ORF11 expression in un-induced and induced BJAB cells harboring vIL-6 deletion mutant strongly suggested that other mechanisms were potentially involved in the regulation of ORF11 expression. Further investigation will be performed to find viral and cellular factors involved in the ORF11 expression and to determine the underlying mechanism of gene regulation.

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