Investigating the Mechanism of Action of a Viral bZIP Transcription Factor

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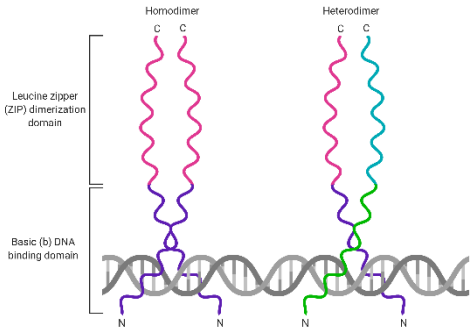
Introduction

The Unfolded Protein Response

Viral infections and endogenous dysregulations in cellular homeostasis cause unfolded proteins to accumulate inside the cell's endoplasmic reticulum (ER), causing ER stress. The cellular unfolded protein response (UPR) constitutes three distinctive arms, the PERK, IRE1, and ATF6 arms, that detect unfolded proteins and restore homeostasis by increasing folding capacity (1).

Kaposi's Sarcoma Associated Herpesvirus

Kaposi's sarcoma associated herpesvirus (KSHV) is a human oncovirus that causes Kaposi's sarcoma (KS) and other cancers (2). During lytic replication, KSHV activates the UPR to aid viral replication, but suppresses the antiviral downstream transcriptional responses aimed at relieving ER stress (3). These downstream responses are governed by basic leucine zipper (bZIP) proteins, which are transcription factors that bind DNA as homo- or heterodimers (4).



bZIP transcription factors bind DNA as homo- or heterodimers. Each bZIP protein contains a leucine zipper (ZIP) and adjacent basic (b) DNA-binding domain that together constitute the bZIP domain (4).

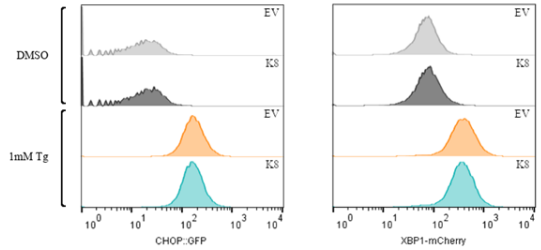
Objective

KSHV encodes a viral bZIP, the multifunctional K-bZIP (also called K8), early during lytic infection (2). Because bZIPs interact and dimerize with one another, we hypothesized that K-bZIP can bind the bZIPs of the UPR to inhibit target gene induction and promote efficient viral replication.

Methods and Results

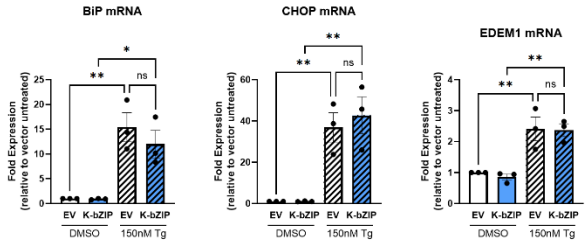
Lentiviruses expressing K-bZIP were generated. CHO-7.1 cells or HEK293A cells were transduced with K-bZIP and treated with thapsigargin to induce ER stress. Expression of UPR-responsive target genes representative of the three arms of the UPR was then assessed using flow cytometry or RT-qPCR.

Flow cytometry analysis confirmed that K-bZIP does not inhibit the PERK and IRE1 arms of the UPR in CHO-7.1 cells.



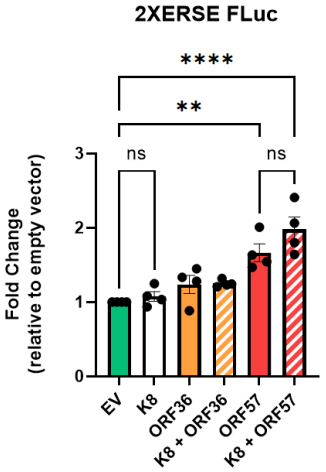
K-bZIP does not inhibit the PERK and IRE1 arms of the UPR. Flow cytometry analysis of dual reporter CHO-7.1 cell line expressing GFP under the ATF4-responsive CHOP promoter or expressing an XBP1s-mCherry fusion protein. CHO-7.1 cells were transduced with empty vector (EV) or K-bZIP (K8) expressing lentiviruses for 72 hours and selected for with puromycin. Cells were then left untreated (DMSO) or treated with 1mM thapsigargin (Tg) to induce ER stress for 20h. CHOP::GFP and XBP1s-mCherry signal was measured by flow cytometry. Data shown for one independent experiment.

qPCR analysis revealed that K-bZIP does not inhibit the PERK, IRE1, and ATF6 arms of the UPR in 293A cells.



K-bZIP does not inhibit the UPR. HEK293A cells were transduced with empty vector (EV) or K-bZIP-expressing lentiviruses for 72 hours. Cells were then left untreated (DMSO) or treated with 150nM thapsigargin (Tg) to induce ER stress for 4h and lysates were harvested for RT-qPCR. Expression of the ATF6-N-induced gene *BiP*, the PERK-induced gene *CHOP*, and the XBP1s-induced gene *EDEM1* were measured by RT-qPCR. Data shown for three independent experiments. Error bars represent SEM. Data was normalized to *GAPDH* mRNA and changes in fold expression were determined using the ΔΔCT method. A two-way ANOVA was done to determine statistical significance (*, p value < 0.05; **, p value < 0.01; ***, p value < 0.001; ****, p value < 0.0001).

Lastly, to assess combinatorial effects of K-bZIP and its known viral interaction partners, ORFs 36, 50, and 57, on the transcriptional activity of the UPR bZIP ATF6-N, HEK293T cells were co-transfected with K-bZIP and interaction partners. ATF6-N activity was measured with an ATF6-N inducible luciferase plasmid.



K-bZIP does not modulate ATF6-N transcriptional activity alone or in combination with known viral interaction partners. HEK293T cells were transfected with plasmids encoding EGFP, EGFP-ATF6N, K-bZIP (K8), FLAG-RTA, HA-ORF36, and HA-ORF57 for 48h and firefly and *Renilla* luciferase relative fluorescent units (RFU) were measured using a luminometer. K-bZIP does not affect ATF6N-mediated transcription of firefly luciferase alone or in combination with viral proteins, but ORF57 alone significantly increases firefly luciferase production. Data shown for four independent experiments. Error bars represent SEM. Fold changes in luciferase expression were normalized to EGFP-ATF6N (EV). A two-way ANOVA was done to determine statistical significance (*, p value < 0.05; **, p value < 0.01; ***, p value < 0.001; ****, p value < 0.0001).

Discussion

K-bZIP did not inhibit the downstream transcriptional responses of any of the arms of the UPR alone or in combination with interaction partners. The functions of K-bZIP during the lytic life cycle of KSHV do not involve UPR modulation.

References

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