BGLF2 INTERFERES WITH CELLULAR MIRNA FUNCTION BY BINDING RISC

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The Epstein-Barr virus (EBV) BGLF2 is a tegument protein with multiple effects on the cellular environment, including induction of SUMO modifications of cellular proteins (SUMOylation) [1]. Using affinity-purification coupled to mass-spectrometry, we identified the miRNA-Induced Silencing Complex (RISC), essential for miRNA function, as a top interactor of BGLF2. We confirmed the BGLF2 interaction with the Ago2 and TNRC6 components of RISC in multiple cell lines and their co-localization in stress granules in some cell backgrounds. In addition, BGLF2 expression led to the loss of processing bodies in multiple cell types, suggesting disruption of RISC function in mRNA regulation. Consistent with this observation, BGLF2 disrupted Ago2 association with multiple miRNAs. Using let-7 miRNAs as a model, we tested the hypothesis that BGLF2 interfered with the function of RISC in miRNA-mediated mRNA silencing. Using multiple reporter constructs with 3'UTRs containing let-7a regulated sites, we showed that BGLF2 inhibited let-7a miRNA activity dependent on these 3'UTRs, including those from SUMO transcripts which are known to be regulated by let-7 miRNAs. In keeping with these results, we found that BGLF2 increased the cellular level of unconjugated SUMO proteins without affecting the level of SUMO transcripts. Such an increase in free SUMO is known to drive SUMOylation and would account for the effect of BGLF2 in inducing SUMOylation. We further showed that BGLF2 expression inhibited the loading of let-7 miRNAs into Ago2 proteins, and conversely, that lytic infection with EBV lacking BGLF2 resulted in increased interaction of let-7a and SUMO transcripts with Ago2, relative to WT EBV infection. Therefore, we have identified a novel role for BGLF2 as a miRNA regulator and shown that one outcome of this activity is the dysregulation of SUMO transcripts that leads to increased levels of free SUMO proteins and SUMOylation. Since RISC mediates the functions of all cellular miRNAs, we expect that the functions of many (or perhaps all) cellular miRNAs will be affected by BGLF2 and may account for the multiple cellular effects of this protein.

1. De La Cruz-Herrera CF, Shire K, Siddiqi UZ, Frappier L. 2018. PLoS Pathog. 7:e1007176.

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