Cyprinid herpesvirus 2 encoded microRNA (miR-KT-635) regulates viral replication by targeting the ORF23

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Abstract

Herpesvirus family have been reported to be able to encode and express functional viral microRNAs that target both viral and cellular transcripts. With previous studies, a novel miRNA miR-KT-635 has been proved to target the viral genes with unknown functions. In this study, the target gene of miR-KT-635 regulated was proved to the viral gene ORF23 directly, the target point on gene sequence was verified and miR-KT-635 was identified to influence the expression of ORF23. According to the bioinformatics analysis, the tRNA domain and ribosome domain in the protein sequence of ORF23 were found to share lots of homology with R2i and P53R2i, which are related to the ribonucleotide reductase small subunit in the host (transform NTP to dNTP). Within expectations, silencing of viral ORF23 or transfecting miR-KT-635 mimics in GiCF could suppress viral propagation significantly. These findings are helpful to understand the effect of virus encoded microRNA on the replication of Cyprinid herpesvirus 2.

Result

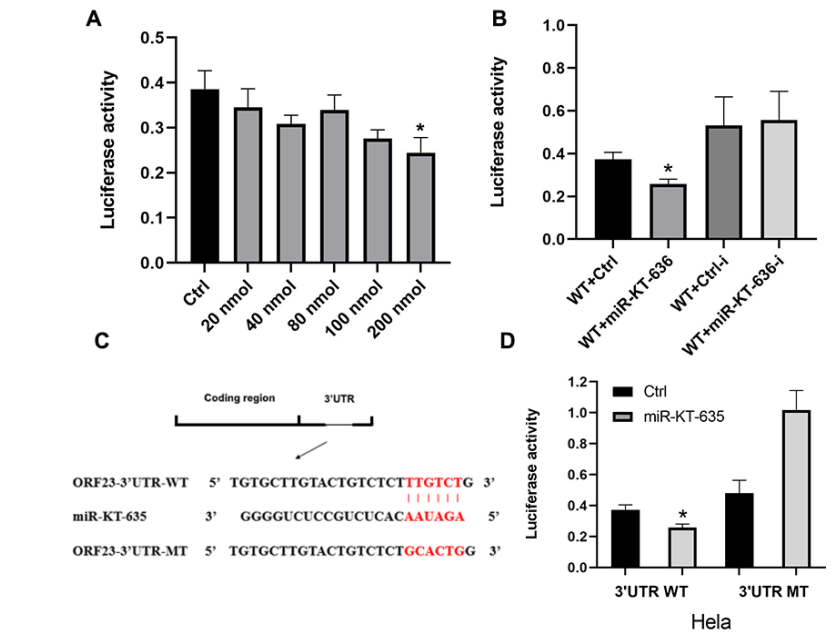

Figure 1. Prediction and verification of miR-KT-635 targeting ORF23（A）HeLa cells were co-transfected with different dose of miR-KT-635 mimics or control miRNA and ORF23-WT. (B) HeLa cells were co-transfected with miR-KT-635 mimics、mimics NC、inhibitor、inhibitor NC and ORF23-WT. Following 48h post- transfection. (C) Sequence of putative binding site of miR-KT-635 within 3′ UTR of ORF23 mRNA. Mutations were introduced to the binding site.（D）HeLa cells were transfected with miR-KT-635 mimics or control miRNA, together with ORF23-WT or ORF23-MT. 48h post-transfection.

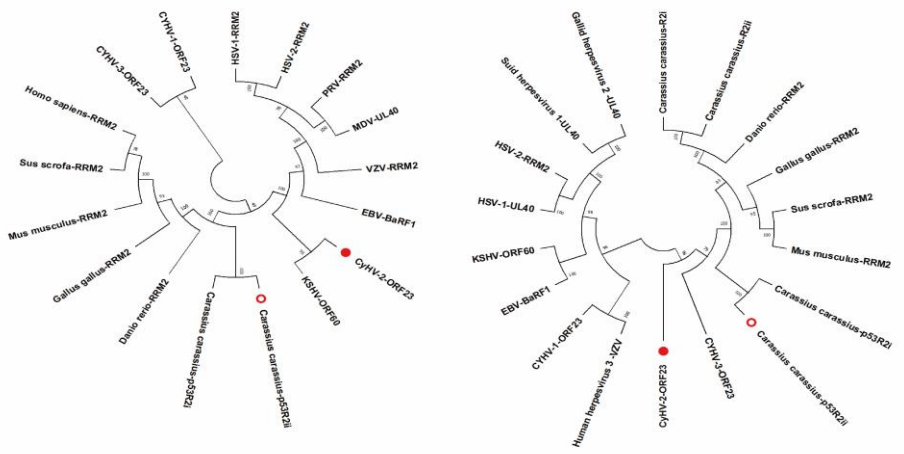

Figure 2. Nucleotide sequence and Amino acid homology analysis by neighbor-joining. O labeled as CyHV-2-ORF23 ● labeled as Carassius carassius-p53R2ii

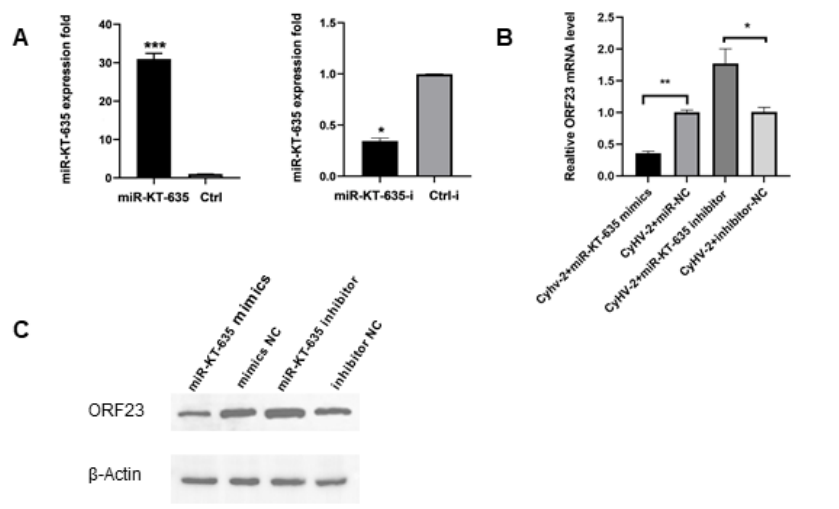

Figure 3. (A) Effects of the miR-KT-635 inhibitor or miR-C12 mimics on miR-KT-635 expression. (B) Effect of miR-KT-635 on ORF23 mRNA expression in CyHV-2-infected GiCF cells. (C) protein levels of ORF23 were also indicated. The miRNA negative control and inhibitor negative control were included in the experiments

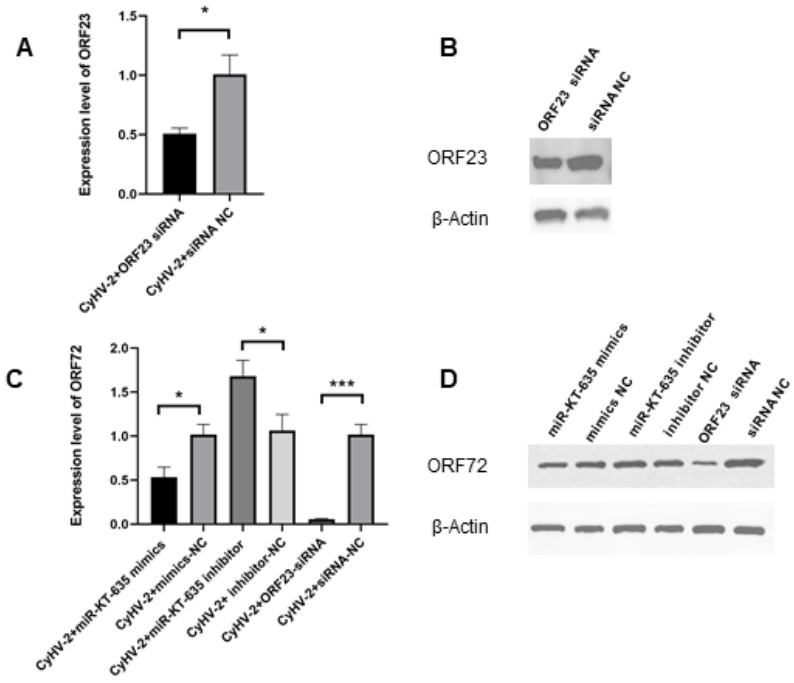

Figure 4. (A)Silencing of ORF23 in GiCF cells by specific siRNA, GiCF cells were transfected with ORF23 siRNA or siRNA NC, ORF23 mRNA levels were detected by qRT-PCR, protein levels by western blot(B). (C) GiCF cells were infected with CyHV-2 and transfected with miR-NC, miR-KT-635 mimics, inhibitor NC, miR-C12 inhibitor, siRNA-NC, or ORF23-siRNA. The infected cells were then collected and subjected to detect CyHV-2 propagation by detecting ORF72 mRNA levels, also verified by protein(D).

Conclusion

- miR-KT-635 was proved to demonstrate significant downregulation of ORF23 expression by binding to its 3'-UTR directly during viral infection.
- In the process of viral infection, transfection of miR-KT-635 mimics reduced the expression of ORF23 mRNA and inhibited the translation of its protein and CyHV-2 of the nucleocapsid genes(ORF72).
- ORF23 may have a similar amino acid sequence to the small subunit of ribonucleotide reductase through bioinformatics analysis, and miR-KT-635 may has an associated effect on virus replication.