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MIR2911 is a potential inhibitor for Nipah virus

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Highlights:

- MIR2911 targets NiV genome via sequence-specific binding.
- Multisite targeting enhances MIR2911-mediated repression *in vitro*.
- Plant-derived miRNA offers candidate antiviral against NiV.

Abstract: Nipah virus (NiV) is a highly pathogenic zoonotic virus for which no approved virus-specific antiviral therapy is currently available. MIR2911, a honeysuckle-derived small RNA, has shown antiviral activity against several RNA viruses. In this study, in silico screening of the NiV Malaysia strain (NiV-M) reference genome identified multiple candidate MIR2911-binding sites, from which a subset of high-confidence sites were selected for experimental validation. Dual-luciferase reporter assays showed that MIR2911 specifically reduced reporter activity driven by NiV target sequences, and this effect was abolished by mutation of the seed-matched regions. A tandem reporter containing multiple NiV target sites showed stronger repression than individual reporters, suggesting that multisite targeting may enhance MIR2911 activity. Although these findings were obtained in a heterologous reporter system that provides only an indirect readout of target function and does not recapitulate the multistep biological processes of authentic viral infection, they suggest that MIR2911 may specifically recognize NiV genomic sequences *in vitro* and justify further evaluation in infection-relevant models.

Keywords: Nipah virus; MIR2911; honeysuckle; cross-kingdom RNA regulation; RNAhybrid target prediction; dual-luciferase reporter validation

1. To the editor

NiV, a zoonotic henipavirus in the family Paramyxoviridae, has an approximately 18.2-kb negative-sense RNA genome encoding six major proteins: nucleocapsid (N), phosphoprotein (P), matrix (M), fusion glycoprotein (F), attachment glycoprotein (G) and the large RNA-dependent RNA polymerase (L). NiV infection can cause severe respiratory disease and encephalitis, with reported case fatality rates ranging from 40% to 75% [1–3]. Although neutralizing antibodies and antiviral candidates such as remdesivir have shown promise in experimental or compassionate-use settings, no NiV-specific therapeutic agent has been globally approved to date [1,4,5]. MIR2911, a small RNA derived from honeysuckle (Lonicera japonica), has been reported to exhibit antiviral activity against several RNA viruses. This plant-derived molecule directly targets influenza A virus genomic RNA and has also been reported to inhibit multiple RNA and DNA viruses, including SARS-CoV-2, porcine reproductive and respiratory syndrome virus (PRRSV) and human papillomavirus (HPV) [6–8]. On this basis, we asked whether the NiV genome contains sequences that could be recognized by MIR2911.

We first screened the NiV-M reference genome using RNAhybrid [9]. This initial analysis identified 156 candidate MIR2911-binding loci. We then applied two additional filters: complete complementarity within the MIR2911 seed region (nucleotides 2–8) and a predicted minimum free energy (MFE) of ≤ −20 kcal/mol. Eighteen high-confidence sites met these criteria (Figure 1), of which 11 were conserved in the representative genome of the Bangladesh lineage of NiV (Supplementary Table S1). These loci were distributed across coding and non-coding regions, including regions associated with N, P, F, and L [10–13], suggesting that MIR2911 may engage multiple viral loci rather than a single hotspot.

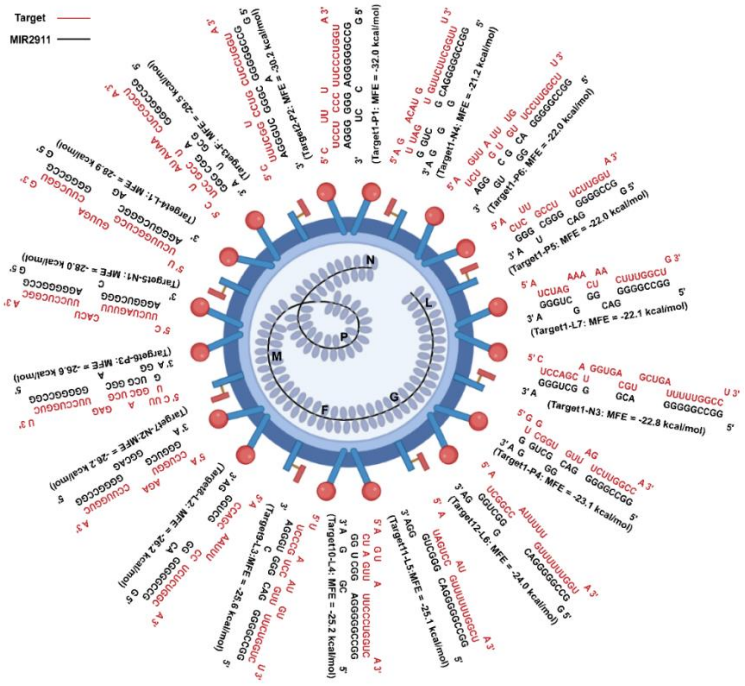

Figure 1. Predicted MIR2911 target sites in the NiV-M genome. Predicted MIR2911-binding sites on the NiV-M genome with complete complementarity in the seed region (nucleotides 2–8) and MFE ≤ −20 kcal/mol.

To determine whether MIR2911 can sequence-specifically repress the predicted NiV sequences, we selected five representative sites for dual-luciferase reporter assays. These sites were chosen because they showed favorable predicted pairing and represented distinct genomic regions. HEK293T cells were then co-transfected with the reporter plasmids and either synthetic MIR2911 or a non-targeting control RNA (ncRNA). Compared with the control RNA, MIR2911 reduced normalized luciferase activity for four of the five wild-type reporters by approximately 20% (Figure 2A). Importantly, this reduction was abolished when the corresponding target sites were mutated (Figure 2B), supporting sequence-specific binding rather than nonspecific effects of transfection.

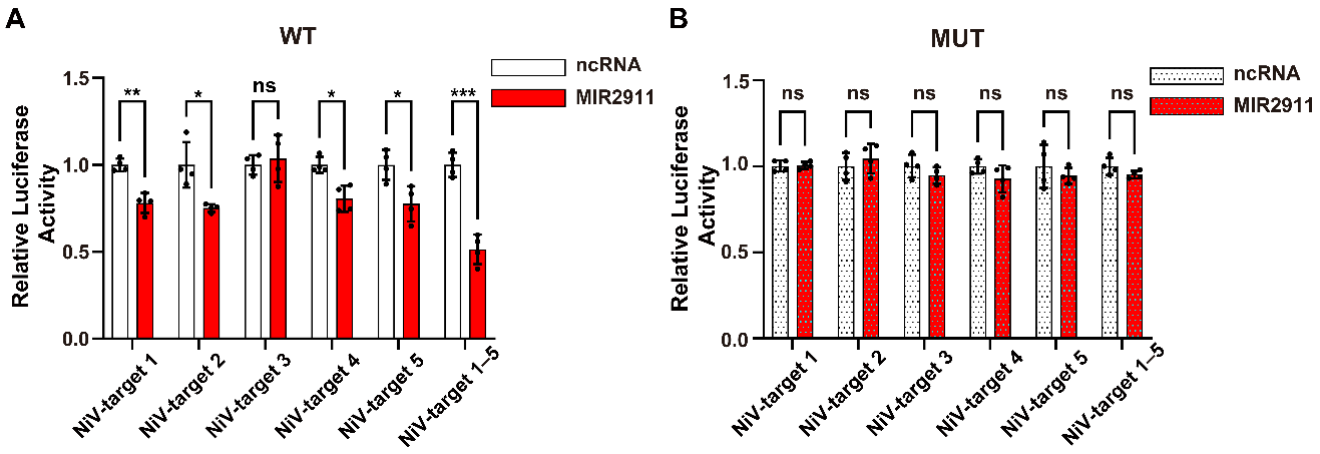

Figure 2. MIR2911 targets NiV-M sequences in a dual-luciferase reporter assay. Dual-luciferase reporter constructs contained individual wild- or mutant-type NiV target sites (NiV-target 1–5) or a tandem reporter containing all five target sites (NiV-target 1–5). HEK293T cells were co-transfected with reporter constructs and MIR2911 or ncRNA, and luciferase activity was measured 36 h later. **(A)** Luciferase activity of wild-type reporter constructs after co-transfection with MIR2911 or ncRNA (n = 4, technical replicates per condition); **(B)** Luciferase activity of the corresponding mutant reporter constructs after co-transfection with MIR2911 or ncRNA (n = 4). Values are shown as mean ± SEM. P values were determined using Student's two-tailed t-test and adjusted using the Holm-Sidak method. *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant.

To assess whether simultaneous targeting of multiple sites could enhance MIR2911-mediated repression, we generated a tandem reporter construct containing all five selected NiV target sites (NiV-target 1–5). Compared with the control, MIR2911 reduced luciferase activity from the tandem reporter to a greater extent than from the individual reporters (Figure 2). In the present reporter system, MIR2911-mediated repression was more pronounced when multiple target sites were present in the same transcript; however, this finding is limited to a heterologous assay and may not reflect the native genomic context of these sites within the viral ribonucleoprotein complex.

Notably, the predicted MIR2911-binding sites are located within genomic regions encoding proteins essential for the NiV life cycle, including the F protein, which mediates viral entry [14,15], the L protein, which drives viral RNA synthesis [13], and the N protein, which encapsidates and protects the viral genome [11]. Collectively, these findings support the possibility that MIR2911 may target NiV sequences in a sequence-dependent manner and provide a foundation for further investigation of its antiviral potential.

At present, treatment of NiV infection remains largely supportive. During the acute phase, intensive care is often required for severe manifestations, including encephalitis and respiratory failure [3,16]. Among investigational therapeutic options, neutralizing antibodies such as m102.4 have been used for post-exposure prophylaxis under compassionate-use conditions [17–19]. Given that honeysuckle-derived MIR2911 has been investigated in other viral systems, the present target prediction and reporter data may inform future preclinical studies, pending infection-relevant validation.

The magnitude of reporter repression observed here was modest, and these data should therefore be interpreted conservatively. The present study demonstrates sequence-dependent repression of NiV-derived reporters in a heterologous reporter system, but it does not establish inhibition of authentic NiV replication. Nevertheless, the combination of multilocus in silico prediction and mutation-sensitive reporter validation provides a rationale for further study. We therefore propose MIR2911 as a candidate small RNA for follow-up antiviral evaluation against NiV.

However, because NiV is a negative-sense RNA virus whose genomic RNA is tightly encapsidated by the N protein within the ribonucleoprotein (RNP) complex throughout its replication cycle, the accessibility of these predicted sites in infected cells may be substantially constrained by nucleocapsid association and RNP architecture [11–13]. This structural constraint is consistent with the broader principle that the antiviral activity of nucleic acid-binding factors depends fundamentally on physical access to viral RNA sequences [20]. Consequently, the in silico-predicted and reporter-validated MIR2911 binding sites may not be freely accessible in the native viral context, underscoring the need to evaluate target accessibility in authentic infection models.

2. Material and methods

2.1. Cell culture

HEK293T cells (Shanghai Cell Bank, Chinese Academy of Sciences, Shanghai, China) were cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM; Gibco, 10564011, MA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, 10099141C, MA, USA), penicillin and streptomycin. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

2.2. Target prediction

The genome sequence used for primary target prediction was the NiV-M reference genome (AF212302.2), retrieved from the GenBank. Potential MIR2911-binding sites were predicted using RNAhybrid. Candidate sites were first screened using an MFE threshold of ≤ –20 kcal/mol and then filtered for complete complementarity within the MIR2911 seed region (nucleotides 2–8). The MFE threshold of ≤ −20 kcal/mol was selected based on empirically established stringency criteria used in prior MIR2911 target prediction campaigns and RNAhybrid-based miRNA screening studies, aiming to retain only duplex predictions with robust thermodynamic stability [21]. Exact genomic coordinates, local target sequences, genomic annotations, and cross-lineage conservation across representative Bangladesh-lineage genomes are summarized in Supplementary Table S1.

2.3. Plasmid construction

Reporter plasmids corresponding to five representative wild-type loci (WT-NiV-target 1–5) were generated by inserting fragments spanning the predicted MIR2911-binding sites (target site ±150 bp) into the 3' untranslated region (3'UTR) of the pmirGLO vector. To assess binding specificity, corresponding mutant constructs (MUT-NiV-target) were generated by replacing the seed-matched target region with a nonbinding sequence in the same sequence context. DNA fragments were synthesized by Vazyme Biotech Co., Ltd. To reduce redundancy in the main text, mutant core sequences are listed in Supplementary Table S2. In addition, a tandem reporter construct containing all five selected MIR2911 target sites (NiV-target 1–5) was generated by concatenating the individual target fragments into the same pmirGLO reporter backbone to evaluate the effect of multi-site targeting. All constructs were confirmed by Sanger sequencing before transfection.

2.4. Dual-luciferase reporter assay

HEK293T cells were seeded in 24-well plates and co-transfected with 0.2 μg of the indicated reporter plasmid and 20 pmol of synthetic MIR2911 or a ncRNA. For multi-site analysis, cells were transfected with either the individual reporters (NiV-target 1–5) or the tandem reporter (NiV-target 1–5) under the same transfection conditions. Total plasmid and RNA inputs were kept constant across all groups. 6 hours after transfection, the medium was replaced with DMEM containing 2% FBS. Luciferase activity was measured 36 h post-transfection, a time point selected based on prior literature and pilot experiments indicating optimal signal-to-noise ratio under our experimental conditions, using the Dual-Luciferase® Reporter Assay Kit (Yeasen, 11402ES60). Firefly luciferase activity was normalized to Renilla luciferase activity (Firefly/Renilla). Each condition was assayed in 4 replicate wells.

Declaration of generative AI and AI-assisted technologies

During the preparation of this manuscript, the authors used generative AI tools only to improve language and readability. Specifically, the authors used ChatGPT for language polishing only in limited sections. The authors take full responsibility for the content of the manuscript.

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Authors' contribution

Supervision, funding acquisition, resources and project administration, Zhen Zhou, Yun Xu and Chen-Yu Zhang; conceptualization, investigation, formal analysis, visualization and writing—original draft, Jieqiong Lei; writing—review and editing, Lijing Xia, Xudong Zhu, Yiting Wu and Zhenbiao Yu. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

Chen-Yu Zhang holds the position of Editor-in-Chief for *ExRNA* and has not peer reviewed or made any editorial decisions for this paper.

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