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MiR-525-3p mediates antiviral defense to rotavirus infection by targeting nonstructural protein 1

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ABSTRACT

MicroRNAs (miRNAs) are short RNAs of approximately 22 nucleotides that post-transcriptionally regulate gene expression by controlling mRNA stability or translation. They play critical roles in intricate networks of host-pathogen interactions and innate immunity. Rotaviruses (RVs) are the leading cause of severe diarrhea among infants and young children worldwide. This study was undertaken to demonstrate the importance of cellular miRNAs during RV (human Wa RV or Rhesus RV) strains infection. Twenty-nine differentially regulated miRNAs were identified during RV infection, and miR-525-3p was downregulated and validated by quantitative real-time polymerase chain reaction (qRT-PCR). MiR-525-3p mimic inhibited RV replication in dose-dependent manner. Correspondingly, the miR-525-3p inhibitors enhanced RV replication. We confirmed that miR-525-3p was complementary to the 3′ untranslated region (UTR) of nonstructural protein 1(NSP1) of RV (Wa or Rhesus) strains. Interestingly, miR-525-3p induced type I interferon (IFN) expression and proinflammatory cytokines during RV infection through IFN regulatory factor (IRF) 3/IRF7 and NF-κB activation, which can induce an antiviral state to further suppress RV infection. In addition, RV suppressed miR-525-3p expression to evade host innate immunity through the action of the RV protein NSP1. These results suggest that miR-525-3p has the potential to be used as an antiviral therapeutic against RV infection.

1. Introduction

Rotaviruses (RVs) are the cause of acute viral gastroenteritis in infants and young children. A member of the Reoviridae family, RV is a non-enveloped double-stranded RNA (dsRNA) virus that encodes 12 viral proteins including six structural proteins (VP1-4, VP6 and VP7) and six non-structural proteins (NSP1-6) [1]. RV infects intestinal host epithelial cells, resulting in cell damage and causing gastroenteritis, which leads to severe diarrhea and sometimes even death from dehydration. Acute diarrhea caused by RV is an important public health problem: RV causes 114 million episodes of diarrhea, resulting in 24 million clinic visits and 2.4 million hospitalizations annually [2]. RV infection results in approximately 500,000 deaths worldwide each year [3]. The availability of two vaccines, Rotarix® (GlaxoSmithKline) and RotaTeq (Merck), have reduced the global burden of disease caused by RV [4,5], but these have not yet been distributed globally, nor are they highly effective in some impoverished settings [6]. Therefore, preventive and therapeutic strategies are urgently required to fight this pathogen.

MicroRNAs (miRNAs) are a class of post-transcriptional regulators comprising non-coding small (~22-nucleotide [nt]) molecule RNA species that regulate specific genes and modulate a range of fundamental cellular processes, including embryonic development [7], cell proliferation [8], metabolic homeostasis [9], and apoptosis [10]. miRNAs are initially expressed as 5′-capped and polyadenylated RNA polymerase II transcripts that are subsequently cleaved by the RNase III enzyme Drosha in the nucleus to produce a hairpin RNA of ~70 nucleotides known as a pre-miRNA [11,12]. Pre-miRNAs are eventually cleaved by the RNase III enzyme Dicer in the cytoplasm to produce ~22-nt double-stranded RNA duplexes. MiRNAs regulate gene

Abbreviations: miRNA, microRNA; RV, rotavirus; qRT-PCR, quantitative real-time polymerase chain reaction; UTR, untranslated region; NSP1, nonstructural protein 1; MOI, multiplicity of infection; IFN, interferon; IRF, interferon regulatory factor; dsRNA, double-stranded RNA; miRISC, miRNA-induced silencing complex; RNAi, RNA interference; EBV, Epstein-Barr virus; SV, Simian virus; JCV, JC human polyomavirus; BKV, BK human polyomavirus; HCMV, human cytomegalovirus; KSHV, Kaposi's sarcoma herpesvirus; HSV-1, herpes simplex virus type 1; MDV, Marek's disease virus; HHV-8, human herpesvirus 8; HIV-1, human immunodeficiency virus type 1; PFV-1, primate foamy virus type 1; VSV, Vesicular stomatitis virus; HCV, hepatitis C virus; ISGs, IFN-simulated genes; OAS1, 2′-5′-oligoadenylate synthetase 1; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; RLR, RIG-1-like receptor; IP, immunoprecipitation; CPE, cytopathic effect; FFA, Fluorescent-focus assay

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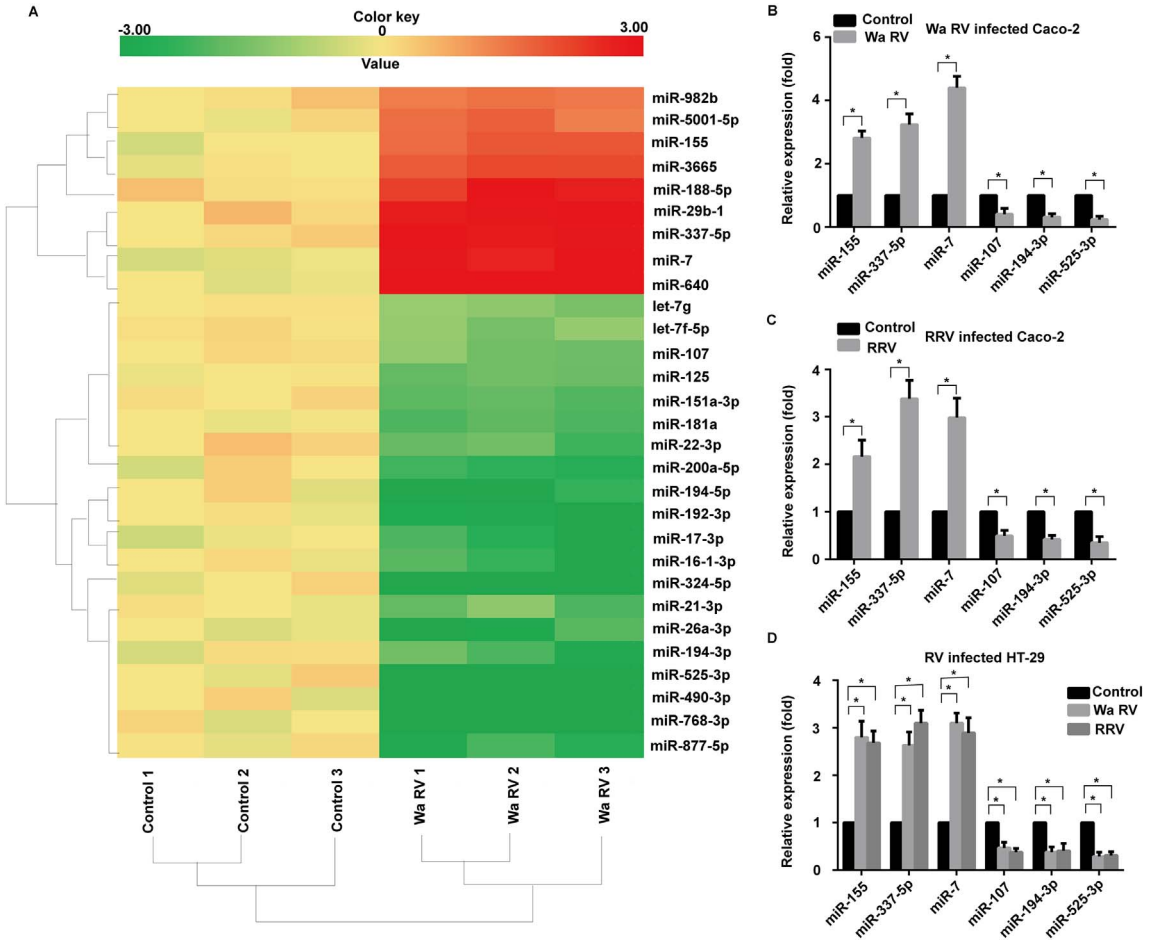

Fig. 1. Differential miRNA expression in RV-infected cells. (**A**) Heat map for differentially expressed miRNAs in Wa RV-infected and mock-infected Caco-2 cells (control). Red indicates an induction and green indicates a reduction of expression in infected cells compared with the mock infected cells, with the color intensity correlating with the level of change. (**B, C**) Caco-2 cells were infected with Wa RV (**B**) and RRV (**C**) strains at an MOI of 2 at 12 h postinfection. The expression of specified miRNAs was analyzed using qRT-PCR and compared with mock-infected cells (control). The fold change in the transcript was calculated by normalizing the relative gene expression to U6 using the formula $2_T^{-\Delta\Delta c}$. (**D**) HT-29 cells were infected with RV (Wa RV or RRV) strains at an MOI of 2 at 12 h postinfection. The expression of specified miRNAs was analyzed using qRT-PCR and compared with mock-infected cells (control). The fold change in the transcript was calculated by normalizing the relative gene expression to U6 using the formula $2_T^{-\Delta\Delta c}$. The data are presented as the mean ± SD of three independent experiments, and each performed in triplicates ($^*P < 0.05$).

expression by pairing with complementary sequences in the 3′ UTRs of transcripts. The actions of miRNAs are mediated by the miRNA-induced silencing complex (miRISC) that uses the mature miRNA guide strand as a template to identify perfectly or partly complementary target mRNAs for cleavage or translational repression. Recent studies have shown that miRNA–mediated RNA interference (RNAi) is a novel and highly evolutionarily conserved mechanism for gene expression regulation at the posttranscriptional level [13]. According to bioinformatics, approximately 1/3 of human genes may be regulated by miRNAs [14]. Over 1000 distinct miRNA genes have been identified in the human genome in the miRBase at the University of Manchester.

Viruses have evolved highly sophisticated gene-silencing mechanisms to evade the host immune response in a hide-and-seek game between the viruses and host. Many reports have shown that viruses encode a miRNA pathway that protects them against the cellular antiviral response. The Epstein-Barr virus (EBV), Simian virus (SV), JC human polyomavirus (JCV), BK human polyomavirus (BKV), human cytomegalovirus (HCMV), Kaposi's sarcoma herpesvirus (KSHV), herpes simplex virus type 1 (HSV-1), Marek's disease virus (MDV), human herpesvirus 8 (HHV-8), and human immunodeficiency virus type 1(HIV-1) encode sets of miRNAs [15–22]. Many studies have focused on direct regulation of viruses by host cellular miRNAs that can target the genetic material of invading viruses. Cellular miR-32 can inhibit the accumulation of primate foamy virus type 1 (PFV-1) in HeLa and BHK 21 cells and play a role in antiviral defense [23]. Dicer knockout mice are more sensitive to Vesicular stomatitis virus (VSV) infection than are wild-type mice because of miRNA synthesis disorders, especially of miR-24 and miR-93, which are complementary to the VSV D protein and L protein [24]. After IFN-β treatment of liver cells, many miRNAs are upregulated, including 8 miRNA (miR-1, miR-30, miR-128, miR-196, miR-296, miR-351, miR-431 and miR-448) that are predicted targets in the hepatitis C virus (HCV) genome. These synthetic miRNAs can decrease the HCV RNA copy number [25]. These studies showed that host cellular miRNAs play an antiviral role by inhibiting the expression of key viral proteins.

RV infects the apical cells of the villi of the small intestine, causing cell death and desquamation. Host miRNAs are closely related to RV infection. Chanda S et al. had confirmed that RV NSP5 up-regulates miR-142-5p, which plays the antiviral function during RV infection [26]. Eukaryotic initiation factor 2 is phosphorylated by RV dsRNA to inhibit the translation of the host protein [27]. RV nonstructural protein 3 (NSP3) interacts with eukaryotic initiation factor 4 gamma and elicits cytoplasmic poly (A)-binding protein (PABP-C1) from translation initiation complexes, ultimately resulting in a global shutoff of host protein synthesis [28,29]. RV can inhibit the type I interferon (IFN) response by nonstructural protein 1 (NSP1)–mediated degradation of IFN regulatory factors (IRFs) or NSP1-mediated repression of NF-κB activation to subvert induction of type I IFN [30,31]. Type I IFN can