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miR-146a facilitates replication of dengue virus by dampening interferon induction by targeting TRAF6

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Summary *Objectives:* To investigate the role of miR-146a in dengue virus (DENV) replication.
Methods: Expression levels of miR-146a were measured by real-time PCR and Northern blot. Role of miR-146a was tested by overexpression and inhibition assays. Real-time PCR and 50% tissue culture infective dose (TCID50) assays were used to detect RNA levels and extracellular yields of DENV respectively. Interferon (IFN) levels induced by DENV infection were measured by real-time PCR and ELISA respectively. IFN-β neutralization and RNAi were used to test the involvement of IFN-β in the effects of miR-146a. TNFR-associated factor 6 (TRAF6) level was measured by Western-blot.
Results: miR-146a expression was significantly increased in primary human monocytes and THP-1 cells upon DENV infection. Overexpression of miR-146a increased DENV2 replication, while inhibition of miR-146a decreased the viral replication. miR-146a impaired the IFN production and the DENV2 replication suppressed by miR-146a inhibition was partially restored by neutralization of IFN-β or depletion of interferon receptor (IFNAR) 1 or 2. Furthermore, miR-146a targets TRAF6 and overexpression of TRAF6 reversed the effects of miR-146a on IFN-β induction and viral replication.

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Conclusions: DENV infection significantly induced the expression of miR-146a, which facilitated viral replication by targeting TRAF6 and dampening IFN-β production.

Introduction

Dengue virus (DENV), a member of family *Flaviviridae*, has a positive single-stranded RNA genome. There are four related but distinct serotypes of DENV, known as DENV1, 2, 3 and 4.[1] Diseases caused by DENV infection, including dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), become the most prevalent arthropod-borne viral diseases in subtropical and tropical regions of the world.[1] According to the World Health Organization report, there are 50 million dengue infections and 500,000 cases of DHF leading to hospitalization each year.[1] Despite its potential for worldwide morbidity and mortality, the immunopathogenesis of DENV diseases remains obscure and no effective treatment or vaccine against DENV is available.

The disease severity of DENV infection has been associated with the rapid initiation of innate host defense, which might be a limiting step of DENV infection. During the critical phase, innate antiviral mechanisms mediated by interferon (IFN) are potentially the most important pathways of the host defense limiting viral replication. The IFN family consists of three subtypes (type I, II and III IFNs). Type I IFNs, including 13 IFN-αs and a single IFN-β, -ω, -ε or -κ, are well-known as antiviral IFNs. Type II IFN, also known as IFN-γ or immune IFN, is expressed in natural killer (NK) or activated T cells.[2] The type III IFN family comprises three subtypes, i.e. IL-28A/B and IL-29, which are co-produced with IFN-β and share many functional characteristics with type I IFNs.[3] Secreted IFNs bind to interferon receptor (IFNAR) 1 or 2 and activate downstream signaling pathways, consequently upregulating the expression of more than 300 interferon stimulated genes (ISGs).[2] Type I and II IFNs have been reported to effectively restrict the propagation of DENV.[4] Although type III IFN is able to prevent the replication of multiple viruses, including hepatitis B virus and hepatitis C virus,[5] pretreatment of type III IFN did not show a significant protection against DENV2 infection in hepatoma HepG2 cells.[6] On the other hand, in order to establish a successful infection and to complete their life cycle, viruses have developed numerous strategies to alleviate or evade the IFN antiviral defense.[7] Clearly, viruses would keep the production of viral pathogen-associated molecular patterns (PAMPs) to a minimum[7] or encode proteins to antagonize the type I IFN signaling by targeting different components of this signaling pathway, such as signal transducer and activator of transcription (STATs).[8] In addition, viruses have developed mechanisms to exploit the host encoded proteins or non-coding RNAs such as microRNAs (miRNAs), for their own benefit. For example, vesicular stomatitis virus (VSV) exploits host encoded miR-146a to decrease IFN production[9] and miR-155 to attenuate IFN signaling[10] to subvert the antiviral immune responses.

miRNAs, a class of highly conserved small non-coding RNAs, function mainly through binding to the 3′-untranslational region (UTR) of target mRNA to induce degradation or suppress its translation.[11] miRNAs play important roles in diverse biological processes, such as development, infection, inflammation and tumorigenesis.[12] Recent evidence revealed that miRNAs such as miR-9,[13] miR-21,[14] miR-125a,[15] miR-132,[16] miR-146a/b,[16] and miR-155[16] are inducible by PAMPs such as lipopolysaccharides (LPS) or poly (I:C). These miRNAs are also engaged in regulating the innate immune responses.[17] Studies show that miR-146a is induced by different viruses or viral products, such as human T-cell leukemia virus type I (HTLV-1),[18] HIV,[19] VSV,[9] Epstein–Barr virus (EBV) latent membrane protein 1,[20] and Kaposi's sarcoma-associated herpesvirus (KSHV) K13 protein.[21] In the VSV model, miR-146a induction leads to an impaired antiviral state via targeting TNFR-associated factor 6 (TRAF6), and IL-1R-associated kinase (IRAK) 1 and 2, which are important molecules in innate immune signaling pathway.[9,16] To the best of our knowledge, the role of miR-146a in DENV infection remains unclear.

Recently, several groups engineered DENV or other Flavivirus by inserting the target sequences of selected miRNAs (e.g., miR-122,[22] miR-124a,[23] miR-128a,[23] miR-142,[24] miR-218[23] or let-7c[23]) into the 3′UTR of virus genome. These miRNAs restrict virus replication[22,24] and modulate the phenotypes of other Flavivirus members[23] by targeting the inserted sequence. For instance, Pham et al. showed that by inserting a hematopoietic specific miRNA (miR-142) target sites into the DENV2 genome, miR-142 restricts its replication in dentric cells (DCs) and macrophages, while has no direct effect on replication in non-hematopoietic cell types.[24] However, whether host miRNAs have an impact on infection of wild-type DENVs remains unknown.

Study presented herein was designed to investigate the role of miR-146a in DENV infection. Our data revealed that DENV infection significantly enhanced the expression levels of miR-146a in primary human monocytes and monocytic THP-1 cells. This DENV-induced miR-146a promoted viral replication partially through impairment of IFN-β in THP-1 cells. In addition, we demonstrated that the proviral effect of miR-146a is mainly dependent on targeting TRAF6. These data suggested that miR-146a might be a vital target for the prevention and clinical treatment of DENV diseases.

Materials and methods

Reagents

Antibodies against TRAF6 were obtained from Santa Cruz (CA) and β-actin antibody was purchased from Sigma–Aldrich (MO). The pUNO vector and pUNO-hTRAF6 plasmids were purchased from Invivogen (CA). IFN-β recombination protein and IL-28A recombination protein were purchased from R&D (MN). Neutralizing anti-IFN-β antibody was purchased from Calbiochem (Germany).

Cell culture

The human monocytic cell THP-1 (ATCC; TIB-202) was cultured in RPMI-1640 (GIBCO) with 10% fetal bovine serum