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Enterovirus 71-induced has-miR-21 contributes to evasion of host immune system by targeting MyD88 and IRAK1

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ABSTRACT

Enterovirus71(EV71), the etiological agent of hand-foot-and-mouth disease, has increasingly become a public health challenge around the world. Type I interferons (IFNs) are an important family of cytokines that regulate innate and adaptive immune responses to pathogens.These pathways are tightly regulated by the host to prevent an inappropriate cellular response, but viruses can modulate these pathways to proliferate and spread. In this study, we demonstrated that EV71 evades the immune surveillance system to proliferate by activating microRNA-21. We demonstrated that EV71 infection upregulates miR-21, which in turn suppresses EV71-triggered type I IFN production, thus promoting EV71 replication. Furthermore, we demonstrated that miR-21 targets the myeloid differentiation factor 88(MyD88) and interleukin-1 receptor-associated kinase 1(IRAK1), which are involved in EV71-induced type I IFN production.

1. Introduction

Enterovirus 71(EV71), a small, non-enveloped, icosahedral RNA virus that belongs to the family *Picornaviridae*, has caused several outbreaks worldwide and often results in severe neurological disorders and high mortality in children. EV71 mainly causes typical hand-foot-and-mouth disease (HFMD) and seriously affected the Asia-Pacific region in recent years (Chang et al., 2016; Pathinayake et al., 2015; Wang and Liu, 2014). The mechanism of EV71 pathogenesis still remains obscure. It is believed that immature or impaired immunity, upon EV71 infection, is associated with increased morbidity and mortality. Intriguingly, EV71 does not facilitate the production of type I interferon(IFN), a family of cytokines involved in controlling viral replication during the initial stages of infection (Pathinayake et al., 2015). Generally, viral infection prevent IFN attacks by the inhibition of IFN synthesis, inactivation of secreted IFN molecules, deletion of IFNs receptors or blockage of IFN signaling (McNab et al., 2015). Consequently, viruses have developed strategies to evade and antagonize the host immune response and resist the antiviral actions of IFN therapy.

Recent studies have shown an important role of microRNAs in sculpting and modulating many levels of the innate immune response. This includes targeting transcripts encoding components of pattern recognition receptors (PRRs) pathways impacting IFN production, targeting transcripts encoding the IFN cell surface receptors and signal transduction proteins to regulate signaling, and targeting IFN-regulated genes (IRGs) directly, to shape the overall IFN response (Forster et al., 2015; Gantier, 2010). MicroRNAs are abundant small non-coding RNAs (ncRNAs), ~19–24 nucleotide nucleotides (nts) in length, which have significant roles in regulating both cellular and viral gene expression (Gottwein and Cullen, 2008; Kim et al., 2009; Skalsky and Cullen, 2010). Currently, the expression of host miRNAs during EV71 infection is the focus of much interest. Accumulating evidences suggested that human miRNAs control EV71 infection or replication, such as miR-146a, miR-296-5p, and miR-1246 (Ho et al., 2014; Xu et al., 2014; Zheng et al., 2013). Moreover, Many miRNAs, including miR-146, miR-155, miR-98, and miR-21, participate in innate and adaptive immune responses (Cheng et al., 2013; Hou et al., 2009; Johansson et al., 2016; Rothchild et al., 2016; Sharma et al., 2015; Wu et al., 2013; Yang et al., 2015; Young et al., 2016). Those meaningful findings suggested that cellular miRNA-viral interaction may serve as novel regulatory mechanism for antiviral therapy.

In the present study, we analyzed the miR-21 expression profile during EV71 infection and found that miR-21 was rapidly upregulated following EV71 infection. Upregulated miR-21 suppressed myeloid differentiation factor 88 (MyD88) and interleukin-1 receptor-associated kinase 1(IRAK1) expression, which subsequently repressed type I IFN effector gene expression and the type I IFN-mediated antiviral response, thereby promoting viral replication. These data suggested that miR-21 might be a vital target for the prevention and clinical treatment of EV71 diseases. Our research has provided new insights into understanding

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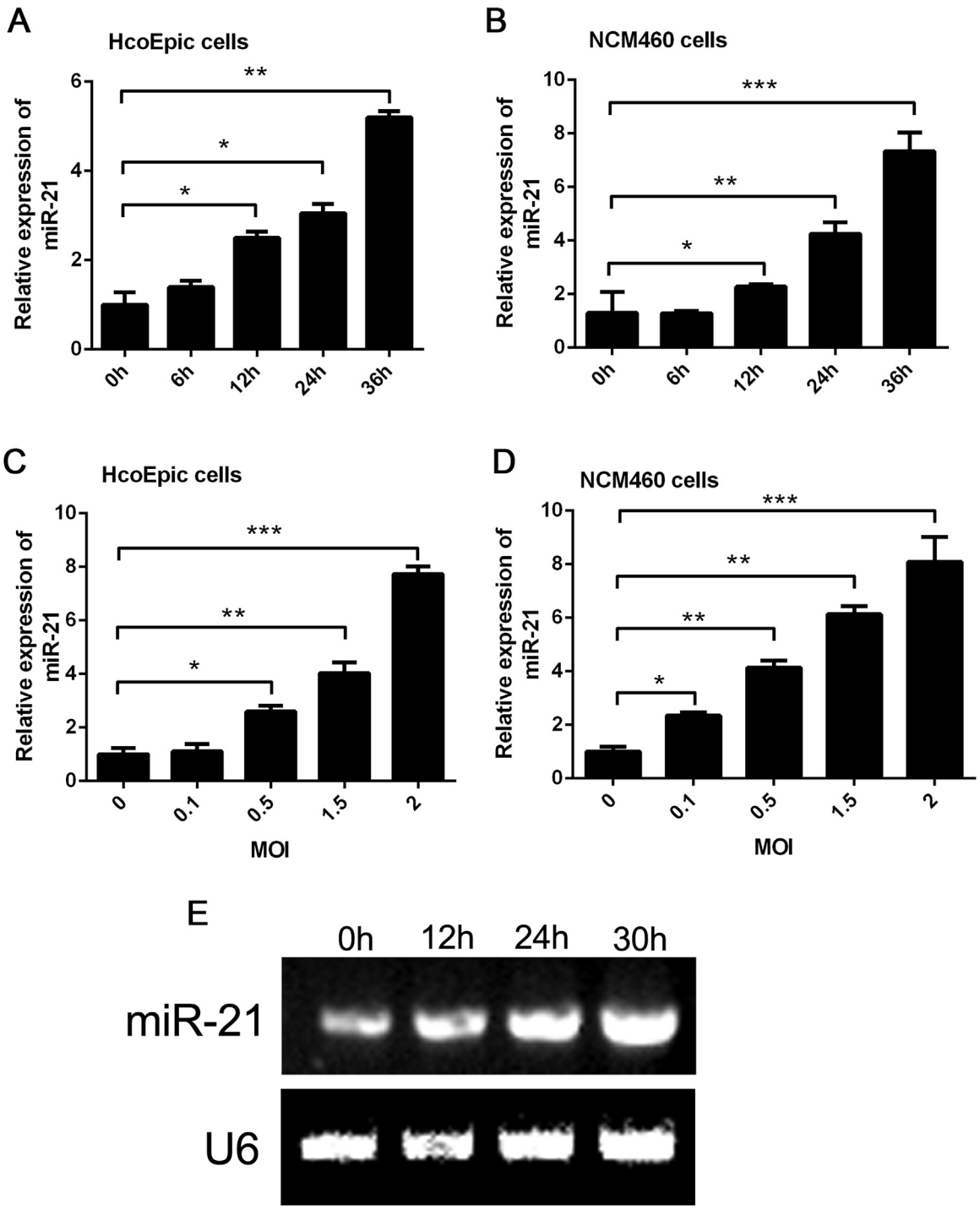

Fig. 1. (A–D), Real-time PCR data to compare miR-21 expression level. Cells were infected with EV71 and harvested for RNA extraction. Expression level of miR-21 was measured by SYBR microRNA assays and normalized to U6. HcoEpic and NCM460 were infected with EV71 at MOI 0.5 at indicated time (A,B) or at indicated MOIs (C,D). (E), Northern blot to compare miR-21 expression level. THP-1cells were infected with EV71 at MOI 0.5 at indicated time, and total RNAs were harvested for Northern blot. Data are shown as mean ± SEM of at least three independent experiments. (*$p < 0.05$, **$p < 0.01$,***$p < 0.001$).

the effects of miRNA on host-virus interactions, and revealed a potential therapeutic target for antiviral intervention.

2. Results

2.1. miR-21 expression was upregulated by EV71 infection

To investigate whether expression of miR-21 was regulated by EV71 infection, HcoEpic and NCM460 cells lines were infected with EV71 respectively and analyzed for miR-21 expression by real-time PCR. Upon EV71 infection, miR-21 expression was gradually induced in HcoEpic cells by 2.3-fold, 2.8-fold and 5.2-fold at 12 h, 24 h and 36 h p.i. respectively, compared to uninfected cells (Fig. 1A). Similarly, in EV71-infected NCM460 cells, miR-21 expression levels was substantially upregulated, by 2.1-fold, 4.1-fold and 7.5-fold higher than the uninfected cells at 12 h, 24 h and 36 h p.i., respectively (Fig. 1B). Moreover, EV71 infection also elicits the miR-21 induction in HcoEpic and NCM460 cells at MOI 0.1, 0.5,1,1.5 and 2 of viral infection (Fig1C