Inhibition of Dengue Virus Infection by Targeting on Macrophage Migration Inhibitory Factor-induced Autophagy

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Abstract

Dengue virus (DENV) infection is the most prevalent mosquito-borne viral infection, of which satisfactory therapeutic drugs are still in need. Previous studies have shown that DENV infection can induce autophagy, which facilitates DENV replication. In addition, the amount of a pro-inflammatory cytokine, macrophage migration inhibitory factor (MIF) in dengue patients’ sera is correlated with the severity of the disease. Since MIF is able to induce autophagy, we propose and test the hypothesis that inhibition of MIF-induced autophagy can inhibit DENV replication. We first showed that DENV infection-induced MIF secretion and autophagy flux in HuH-7 cells. Either suppression of endogenous MIF by short hairpin RNA (shRNA) or inhibition of MIF activity by MIF inhibitors (iso-1 and p425), attenuated DENV infection. On the other hand, co-culture with recombinant MIF enhanced DENV infection and increased viral load through autophagy dependent manner. According to pathogenesis of MIF, we found autophagy as such as minocycline (Mino) could attenuate DENV infection by blocking DENV-induced MIF secretion and autophagy. Furthermore, treatment of minocycline can attenuate DENV infection and viral load both in vivo and in vitro. Taken together, these results support our hypothesis that MIF is a potential therapeutic target and inhibition of MIF by minocycline may represent an alternative therapy approach against DENV infection.

Specific aims and flow chart

1. To elucidate the pathogenic role of MIF on DENV replication and infection.
2. To explore the correlation of MIF and autophagy during DENV infection.
3. To repurpose of antibiotic, minocycline, against DENV infection.

Result 1.

Fig 1. DENV infection induced MIF expression and secretion in HuH-7 cells. HuH-7 cells were either Mock-infected, infected with DENV 2 (m.o.i=3) or UV-inactivated DENV 2 for different time periods as indicated. (A) The MIF and DENV N55 mRNA levels were determined using RT-PCR and quantified after normalization to the corresponding β-actin levels by image J. (B) The secretion of MIF in culture supernatant at different time points were determined by MIF-quantitative sandwich ELISA. All data are presented as the means±SD from at least triplicate independent experiments, and *P<0.05, **P<0.01, ***P<0.001, ns indicates no significance.

Result 2.

Fig 2. MIF induced autophagy formation in HuH-7 cells. HuH-7 cells were treated with recombinant MIF (10, 20 ng/ml) or denature MIF (ΔMIF) in the presence or absence of MIF inhibitor, ISO-1 (50 μM), p425 (10 μM) for 3 h (A) LC3 conversion, which represents autophagosome formation, was determined using Western blotting and quantified after normalization to the corresponding β-actin levels by image J. (B) eGFP-LC3 plasmid transfected HuH-7 cells were treated with MIF or ΔMIF in the presence or absence of ISO-1 or p425 for 3h. After fixation, LC3 puncta were taken by confocal microscope.

Result 3.

Fig 3. Autophagy is critical for DENV replication and infection in HuH-7 cells. (A) LC3 and GFP knock down stable HuH-7 cells (shLC3 and shGFP) were infected with DENV 2 (m.o.i=2) or Mock infected for 36 h. N53 expression, a DENV replication marker, and LC3 conversion were determined using Western blotting. (B) The viral titers in culture supernatant from (A) were determined by focus forming assay (FFA). (C) HuH-7 cells were infected with DENV 2 (m.o.i=2) in the presence or absence of either autophagy inhibitors (NAC, 3-MA) or autophagy inducer (rapamycin, Rapal) for 36 h. The viral titers in culture supernatant were determined by FFA. All data are presented as the means±SD from at least triplicate independent experiments, and *P<0.05, **P<0.01, ***P<0.001, ns indicates no significance.

Conclusion

1. MIF plays pathogenic role on DENV replication.
2. DENV infection induces MIF secretion which facilitates viral replication through autophagy.
3. MIF is a potential therapeutic target and inhibition of MIF by minocycline may represent an alternative therapy approach against DENV infection.