Deciphering the Mechanism of a Host Cellular Factor High Mobility Group Box1 Protein in Dengue Virus Pathogenesis

Dengue disease is a highly prevalent mosquito-borne infection-causing millions of infections annually. The global rapid emergence and distribution of dengue in tropical and sub-tropical regions are exposing almost half of the world's population to the threat. Dengue virus (DENV) causes the overexpression and secretion of many proinflammatory cytokines, including high-mobility group box-1 protein (HMGB1). HMGB1 is the most abundant, extremely conserved, ubiquitously expressed nuclear protein that executes the localization-dependent function. HMGB1 translocation and secretion have been implicated in many chronic infections, metabolic disorders, cancer, and viral and inflammatory diseases. In this study, we explored the contribution of HMGB1 in dengue virus replication in A549 cells. Results showed that HMGB1 is translocated and secreted out during dengue infection. Moreover, blockage of HMGB1 release using ethyl pyruvate resulted in enhanced dengue replication and the knockdown of HMGB1 abolished viral replication. *In-silico, in-vitro* assays, and co-immunoprecipitation revealed the binding of HMGB1 to both untranslated regions of viral RNA. This interaction further induces the expression of proinflammatory cytokines like TNF-α, IL-6, and IL-1β which contributes to the severity of DENV disease. Therefore, our study suggests that the DENV tweaks HMGB1 translocation and its interaction with the DENV genome to stimulate proinflammatory cytokines expression in A549 cells.

DENV exploits various cellular pathways including autophagy to assure enhanced virus propagation. The mechanisms of DENV-mediated control of the autophagy pathway are largely unknown. Our investigations have revealed a novel role for HMGB1 protein in the regulation of the cellular autophagy process in the DENV-2 infected A549 cell line. While induction of autophagy by rapamycin treatment resulted in enhanced DENV-2 propagation, the blockade of autophagy flux with bafilomycin A1 suppressed viral replication. Furthermore, siRNA-mediated silencing of HMGB1 significantly abrogated DENV-induced autophagy, while LPS-induced HMGB1 expression counteracted these effects. Interestingly, silencing of HMGB1 showed a reduction of BECN1 and stabilization of BCL-2 protein. On the contrary, LPS induction of HMGB1 resulted in enhanced BECN1 and a reduction in BCL-2 levels. This study shows that the modulation of autophagy by DENV-2 in A549 cells is HMGB1/ BECN1 dependent. In addition, glycyrrhizic acid (GA), a potent HMGB1 inhibitor suppressed autophagy as well as DENV-2 replication. Altogether, our data suggest that HMGB1 induces BECN1-dependent autophagy to promote DENV-2 replication. Therefore, we postulate HMGB1 as a crucial host element promoting viral propagation and must be considered as an alternative approach for targeting DENV infection. The complex pathogenesis of DENV infection can be better resolved by understanding the complicated correlation between the DENV virus and its host factors.

Growing pieces of evidence demonstrate the importance of metabolic control on proinflammatory response, however, the regulatory mechanism is not known. Here, we suggest a novel mechanism for pyruvate kinase M2 (PKM2) in mediating HMGB1 release during DENV infection. Results showed that the PKM2 expression and nuclear localization were increased in the DENV-2 infection group. Inhibition of PKM2 nuclear translocation by DASA-58 and ML-265 results in decreased HMGB1 release in the supernatant. Furthermore, we observed that DASA-58 and ML-265 resulted in reduced viral protein expression and autophagy. Altogether, our results shed light on an important process for metabolic

regulation of proinflammatory response by controlling HMGB1 release and emphasize the role of a metabolic enzyme in understanding the DENV disease pathogenesis.

Collectively, we have deciphered the proviral mechanism of HMGB1 in DENV virus replication by its interaction with the viral genome to induce a proinflammatory response and inducing autophagy. Furthermore, we unraveled that release of HMGB1 during DENV infection is controlled metabolically by a glycolytic enzyme PKM2 expression and its nuclear translocation. Therefore, the molecular mechanisms associated with host factors that regulate inflammation must be further explored to understand the pathogenesis of severe DENV disease.