West Nile Virus: Therapeutic Strategies for Curing Diseases

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West Nile virus (WNV) is a single-stranded RNA virus and a member of the *Flaviviridae* family. WNV infection induces brain encephalitis and leads to fatality in human as well as animal. The outbreak of the West Nile Virus (WNV) on September 1999 in New York City area is the first incident in Northeast America. As of October, 2005 avian, animal or mosquito WNV infections have been reported from almost of all US states. In human case, 2,435 cases of human diseases and 372 presumptively viremic blood donors were reported. Since avian species including migrating birds are the major host of the virus and the pathogen can be carried by mosquitoes, West Nile Virus outbreak could be widely transmittable and an serious epidemic. Currently, no obvious specific treatment is available. Therefore, we investigated a WNV pathogenesis induced by a capsid (Cp) protein to search novel therapeutic targets for prevention and treatment strategies. A WNV capsid interacting protein (CIP) was identified by yeast-two-hybrid system in mammalian brain library. To characterize biophysiological effect of CIP on WNV, an Adenovirus(AdV)-CIP and a stable cell line NIH3T3-CIP were prepared. Prior to infect WNV, the AdV-CIP was transduced into 293T cell. The NIH3T3-CIP was directly infected with WNV. Four days after infection, cell lysates and tissue culture supernatants were saved for Caspase-3 assay and plaque forming assay, respectively. To verify that the effect is specific for Cp and CIP, two small interfering RNA (siRNA) for Cp and a siRNA for CIP were fused to red fluorescent protein (RFP) and stable cell lines are being established. Apoptosis induced by WNV infection was significantly reduced by CIP over-expression by the AdV-CIP and the stable cell line as determined by Caspase-3 assay. To determine whether the Cp degradation by CIP affects virion encapsidation and further progeny release, harvested tissue culture supernatants were assayed for plaque formation. The progeny production was significantly reduced in tissue culture supernatants harvested from the AdV-CIP treated and the stable cell line. Previously, WNV Cp was reported as a cytopathogenic protein inducing apoptosis and inflammation in vitro as well as in vivo. To determine whether Cp-specific siRNAs can interfere WNV replication, translation or its pathogenicity, several reagents such as chemically synthesized siRNA duplexes, siRNA-RFP plasmids, or stably siRNA transformed cell lines were analyzed. The level of interference was evaluated by Cp-induced apoptosis as determined by Caspase-3 assay and viral progeny production as determined by plaque formation assay. Moreover, degree of WNV RNA replication was

determined by RT-PCR for Cp gene. Apoptosis in siRNA treated and then WNV infected cells was decreased about 50% compared to that of non-infected cells. To determine whether this inhibition is Cp gene-specific, RT-PCR with total RNA was performed. Data also presented that Cp message expression was about 50% decreased. A reduction of viral progeny release is under investigation using plaque formation assay. Our data is the first report that the specific cellular protein suppresses WNV progeny production and this could be a potential therapeutic target for prevention and curing WNV induced brain encephalitis. Moreover, siRNA targeting Cp gene maybe a potential preventive and further protective therapeutics for WNV-induced brain encephalitis.