BROAD-SPECTRUM THERAPEUTICS FOR VIRAL DISEASES: A MEDICAL COUNTERMEASURE PLATFORM FOR EMERGING THREATS

Developing Of Sirna Therapeutic Platform For Immediate Respond Against Emerging Viral Pathogens

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Since the WHO declared eradication of smallpox in 1980, the vaccination for poxvirus has been discontinued. With cessation of vaccination, possibilities of re-emergence or biological weapons by smallpox or other poxviruses have been constantly concerned. Now, the outbreak of monkeypox infection worldwide, smallpox like events, has threatened us. Here, we developed RNAi therapeutic platform for rapidly handle with infection crisis using the variola virus as substitute vaccinia virus. In previous study, we designed 71 siRNAs that target viral genes related to replication in early-stage transcription after infection. Among them, we selected 7 siRNA candidates of the most efficient siRNAs by plaque reduction screening. For enhancement serum stability, siRNAs were modified with methylation on pyrimidines of either strand or both. Generally, modifications on the sense strand are more favorable to increase stability and maintain in vitro efficiency. We evaluated the efficacy of the modified siRNA candidates in vivo. The A5R with sense strand modification showed the highest survival rate at 60% in mice challenged with vaccinia virus. In addition, we designed asymmetric siRNAs (asiRNAs) that can reduce off-target effect by passenger strand. (16+2) and (17+2) asiRNAs which showed virus replication reduction as much as original siRNA were further modified of phosphorothioate and vinylphosphonate for increasing siRNA incorporation into RISC complex and serum stability. Modified asiRNAs showed more plaque reduction than the A5R sense modification. About 90% of A5R mRNA inhibition was observed in vitro when modified (17+2) asiRNA was treated in vaccinia virus infected cells. For targeting to extrahepatic tissues and improving delivery efficiency, we conjugated docosanoic acid (DCA) to modified asiRNA. Modified asiRNA with DCA are intranasally administered into mice at the 3 x 5 mg/kg dose. We evaluated virus titer by plaque reduction and found the virus titer was reduced by treatment DCA-asiA5R than scramble control. Through this study, we established an siRNA platform from target gene selection to modifications for increasing efficacy in vivo. It demonstrated our technology can rapidly apply any types of infectious threats like monkeypox in a few months.