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Adenovirus-mediated shRNA interference against HSV-1 replication *in vitro*

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Background: The UL29 and UL28 proteins encoding by Herpes simplex virus type 1 (HSV-1) are respectively critical for its replication and package. Research has demonstrated that synthesized siRNA molecules targeting UL29 gene could suppress HSV-2 replication and the UL28-null HSV-1 gene can't form infectious virus *in vitro*. Whether the UL28 and UL29 gene silenced by RNAi could be a proper antiviral target of HSV-1 are still unknown.

Methods: Three kinds of recombinant adenoviruses carrying CMV-U6 compounded promoter driven shRNAs targeting UL28 or/and UL29 gene of HSV-1 were constructed, and one kind of recombinant adenoviruses carrying (-)shRNAs targeting none of genome of HSV-1 were constructed as control. After infection of different Ad-shRNAs at a MOI of 100 24 h in advance, the Vero cells in four groups were challenged with HSV-1 at a MOI of 1, and 24 h later, their effects on inhibiting HSV-1 replication were measured by plaque assays forming ability, the transcription level of viral RNA and the protein expression.

Results: The shRNAs delivered by recombinant adenovirus could induce a significant inhibition of viral DNA replication and protein synthesis in Vero cells. The Ad-UL28shRNA, Ad-UL29shRNA, Ad-UL28-UL29shRNA groups suppressed HSV-1 replication by 8-10 folds, 20-22 folds, 35-40 folds respectively (P0.05, compared with Ad-(-)shRNA). The transcription level of viral RNA and the expression of UL28 UL29 proteins also decreased in Ad-shRNAs infected cells.

Conclusions: UL28 and UL29 gene silencing mediated by RNAi technique display comparable antiviral activities against Herpes Simplex Virus *in vitro*. The adenovirus based shRNAs probably will be an alternative strategy for controlling HSV-1 infection.

Keywords: HSV-1; RNAi; shRNA; recombinant adenovirus

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