eGFP-R65 on activation of NF-κB as well as on protein expression of NF-κB P65 in HeLa cells.

Methods The synthesised ribozyme gene targeting NF-κB was inserted into the plasmid pFB-CMV-eGFP with definite direction, and packaged into the recombinant adeno-associated virus serotype 9 by three plasmids co-transfection, then recombinant adeno-associated virus was purified by cesium choride density centrifugation. The purity of recombinant virus rAAV9-eGFP-R65 was observed and verified by transmission electron microscopy and SDS-PAGE and viral tite was checked by GFP. Finally, HeLa cells were infected by the recombinant adeno-associated virus, the activation of NF-κB and protein expression of P65 were analysed by electrophoretic mobility shift assay [1] and Western blot.

Results The high expression of green fluorescence protein expression in HEK293 and HeLa cell lines were found under fluorescent microscope. Electron microscopy and SDS-PAGE test indicated that recombinant adeno-associated virus rAAV9-eGFP-R65 was successfully constructed, and the titre of the virus reached 4.63×10^{12} vg/ml. Western blot and EMSA showed that the protein expression of P65 and the activation of NF-κB in HeLa cells were markedly inhibited after transfection with rAAV9-eGFP-R65.

Conclusions Activation of NF-κB and expression of NF-κB p65 protein in HeLa cells are effectively inhibited by recombinant adeno-associated virus rAAV9-eGFP-R65, which lays the foundation for further researches into therapy nuclear factor-κ B related ischemic diseases.

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CONSTRUCTION OF RECOMBINANT ADENO-ASSOCIATED VIRUS SEROTYPE 9 WITH RIBOZYME GENE TARGETING NF-κB AND ITS SUPPRESSION OF NF-κB ACTIVITY IN HELA CELLS

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Objectives To construct the recombinant adeno-associated virus serotype 9 containing ribozyme gene (R65) targeting nuclear factor-κ B (NF-κB), and investigate the inhibitory effect of rAAV9-