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ANALYSIS OF DIFFERENCES OF EXPRESSION EFFICIENCY ABOUT RAAV9 VECTOR WITH DIFFERENT PROMOTERS IN RATS MYOCARDIAL CELLS

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Objective To compare the expression efficiency and the effect on cell growth about recombinant adeno-associated serotype 9 vector with different promoters in H9C2 cells. We aim to examine whether it can be a foundation for further research of rAAV9 vector used for the gene therapy of ischemic heart disease.

Methods rAAV9-CMV-eGFP and rAAV9-CBA-eGFP were transfected into H9C2 cells at same multiplicities of infection ($\text{MOI}=1 \times 10^7$), respectively. Enhanced green fluorescent protein (eGFP) expression in the cells was observed under inverted fluorescence microscope, and the eGFP-positive cell percentage determined by flow cytometry. The expression of eGFP was quantitated by Western blot. Alamar Blue assay was used to assess the proliferation of the transfected cells.

Results The transduction efficiency of rAAV9-CBA-eGFP and rAAV9-CMV-eGFP in H9C2 cells was $(92.5 \pm 1.0)\%$ and $(74.2 \pm 1.3)\%$ ($p < 0.05$), respectively. eGFP expression of rAAV9-CBA-eGFP is stronger than rAAV9-CMV-eGFP. Alamar Blue assay did not reveal significant differences in the absorbance between the transfected cells and the control cells after transfection.

Conclusion The CBA promoter directs the higher expression from an rAAV9 vector than the CMV promoter in the H9C2 cells without causing cell growth inhibition.