Genetics and Molecular Research

Construction of recombinant adenovirus Ad-rat PLCg2-shRNA and successful suppression of PLCg2 expression in BRL-3A cells

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Genet. Mol. Res. 15 (2): gmr.15028029
Received November 9, 2015
Accepted January 22, 2016
Published May 23, 2016
DOI http://dx.doi.org/10.4238/gmr.15028029

ABSTRACT. Phospholipase Cg2 (PLCg2) induces apoptosis of immune and tumor cells; however, it remains unclear whether PLCg2 promotes hepatocyte apoptosis during liver regeneration (LR). Therefore, to establish a framework for further exploring the function of PLCg2, we generated recombinant adenoviruses carrying a template encoding short hairpin (sh)-RNA targeting PLCg2 (Ad-PLCg2-shRNA), which were used to silence the expression of PLCg2 in BRL-3A cells. First, three pairs of PLCg2-shRNAs were designed, synthesized, and cloned into a shuttle vector, pHBAd-U6-GFP, after annealing. The recombinant shuttle plasmids were co-transfected with the backbone vector pHBAd-BHG into HK293 cells to package the recombinant Ad-PLCg2-shRNAs used to infect BRL-3A cells. Infection efficiency was monitored by observing the number of GFP-positive cells under a fluorescent microscope. To determine the recombinant adenoviruses

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with the highest silencing efficiency, levels of *PLCg2* mRNA were evaluated by qRT-PCR. DNA sequencing confirmed that the correct shRNA coding sequences were inserted into the shuttle vectors and adenoviral plasmids. The titers of three recombinant adenoviruses were at least 1 x 10^{10} PFU/mL. The most effective adenoviral construct, with interference efficiency of 77%, was determined by qRT-PCR. These results show that a recombinant adenovirus, Ad-PLCg2-shRNA, was developed and was effective at silencing the rat *PLCg2* gene. This construct may contribute to the study of PLCg2 in hepatocyte apoptosis during LR.

Key words: Phospholipase C gamma 2; Short hairpin RNA (shRNA); Recombinant adenovirus; BRL-3A cell; Rat

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