Design, construction and identification of siRNA eukaryotic expression vectors targeting to Cdc2/cdk1 and survivin

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View from specialist: It is creative, and of certain scientific and educational value.

[ABSTRACT] Objective: To design and construct siRNA expression vectors, and to identify them. Methods: The primary structures of Cdc2/cdk1 and survivin cDNA were found in GenBank. Then the structure analyses were performed according to the strategy of RNAi, which determined the specific sequences to design siRNA plasmid. Two sequences of Cdc2/cdk1 (phU6-cdc2/cdk1-shRNA1and phU6-cdc2/cdk1-shRNA2) and survivin (phU6-survivin-shRNA1 and phU6-survivin-shRNA2), involved in fluorescein gene were synthesized based on the specific base sequence. Plasmid control pU6- HK-shRNA-a random sequence was also constructed. Cell line CNE2 was treated daily with these different vectors. The phU6-cdc2/cdk1-shRNA1, phU6-cdc2/cdk1-shRNA2, phU6-survivin-shRNA1 and phU6-survivin-shRNA2 were identified by gene base sequencing and were identified by electrophoresis. After administration of phU6-cdc2/cdk1-shRNA1, phU6-cdc2/cdk1-shRNA2, phU6-survivin-shRNA1 and phU6-survivin-shRNA2, fluorescence expression was detected by confocal fluorescence microscopy. After transfection of phU6-cdc2/cdk1-shRNA1, phU6-cdc2/cdk1-shRNA2, phU6-survivin-shRNA1 and phU6-survivin-shRNA2 48 h, the expression inhibition rate was detected by Western blotting. Results: There was a 400 bp balteum in phU6-cdc2/cdk1-shRNA1, phU6-cdc2/cdk1-shRNA2, phU6-survivin-shRNA1 and phU6-survivin-shRNA2, and phU6-HK-shRNA after cut by SalI, which was identical with the size of the objective gene. The gene sequence of phU6-cdc2/cdk1-shRNA1, phU6-cdc2/cdk1-shRNA2, phU6-survivin-shRNA1 and phU6-survivin-shRNA2 was identical with the anticipation. Many cells gave out fluorescent. After transfection of phU6-cdc2/cdk1-shRNA1, phU6-cdc2/cdk1-shRNA2, phU6-survivin-shRNA1 and phU6-survivin-shRNA2 48 h, the expression inhibition rate was 33.00±1.41 and 38.31±1.01 for surviving and 36.33±1.21 and 43.26±1.02 for cdc2/cdk1 at survivin and cdc2/cdk1 protein level detected by Western blotting, respectively. Conclusions: siRNA targeting to expression vectors have been successfully established. All of these new vectors can be effectively transfected into CNE2 cells. The inhibitory rate of phU6-cdc2/cdk1-shRNA2 and phU6-survivin-shRNA2 is higher than that of phU6-cdc2/cdk1-shRNA1 and phU6-survivin-shRNA1.

[KEY WORDS] siRNA; Cdc2/cdk1; survivin; CNE2