

GW23-e0851

STUDY ON THE BIOLOGICAL FUNCTIONS OF P21-ACTIVATED KINASE 5

doi:10.1136/heartjnl-2012-302920a.271

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Objectives PAK5 is a member of P21-activated kinase (PAK) family, which characterised by a highly conserved amino-terminal Cdc42/Rac interactive binding (CRIB) domain and a carboxyl terminal kinase domain. However, the role of PAK5 in apoptosis remains largely unknown. In this study, we constructed pEGFP-C1-PAK5 and established a stably transfected cell line and the effect of PAK5 on cell apoptosis was examined. The results suggest that PAK5 could inhibit the apoptosis induced by staurosporine.

Methods A. Materials: All the materials used in this study were listed in Table 1. (B) Designation and synthesis of primer: Primers target to PAK5 gene were designed according to GenBank. Forward primer 5'-GAAGATCTATGTTTGGGAAGAAAAG-3' with addition of BglII restriction enzyme recognition site and reverse primer: 5'-CGGAATTCTCAGTGATGCCTGTA TTG-3' with EcoRI addition of site. (C) Acquisition of PAK5 Coding sequence: We performed RT-PCR (reverse transcription PCR) using total RNA extracting from People fresh placental tissue. Then we used cDNA library as template and the primers to make PCR. Full-length PAK5 cDNA coding sequence (CDS) was cloned. The length is 2160 bp. (D) Construction of eukaryotic expression vector of PAK5 and DNA sequencing. We used T4 DNA ligase to connect the PCR products and pEGFP-C1 plasmid which had both been cut by restriction enzyme EcoRI/BglII. After transforming it into competent cells *E. coli* DH5 α and screening of kanamycin, we picked the positive cloning colony to extract plasmid DNA and made a 0.8% gel electrophoresis identification after EcoRI/BglII cleavage, sequencing by Sangon Ltd. of Shanghai. (E) Establishment of stably transfected cell line: The recombinant plasmid pEGFP-C1-PAK5 was transfected into hela cells by lipofectamine. Then we cultured the transfected cells by G418 (700 mg/l) for 6 weeks 48 h later and propagated hela cells by limited dilution method. Therefore, a stably transfected cell line named hela-PAK5 was established. (F) MTT: We prepared four parallel groups: a: hela cells, b:hela cells, c: hela-PAK5 cells, d:hela-PAK5 cells. Then we added staurosporine into group b and group d after 24 h cultivating. About 5 h later, we added MTT into each group and got OD values of each group by using Microplate Reader.

Results (A) Identification of eukaryotic expression vector. We got two bands by electrophoresis after EcoRI and BglII restriction enzyme cleavage. As showned in Fig 1, a clear band about 2100 bp in agrose gel can be seen as expected according to the gene sequence. The other band is about 4700 bp which is the expression vector pEGFP-C1. It suggests that PAK5 gene was introduced into pEGFP-C1. (B) DNA sequence analysis: After sequencing analysis

of recombinant plasmid of pEGFP-C1-PAK5, we confirmed that the inserted fragment was PAK5 gene by BLAST. The result of DNA sequence is showed in figure 2. (C) Application MTT method for hela—pak5 cells survival: The cells with staurosporine compared with the control group are different, and the apoptosis are detected ($p<0.05$). While the hela-PAK5 cells with staurosporine compared with the control group cells are the same. It is shown that the hela-PAK5 cells can resist apoptosis ($p>0.05$) (figure 3).

Conclusions In our study, we constructed the eukaryotic expression vector pEGFP-C1-PAK5 successfully and transfected the PAK5 gene stably into hela cells. And the experiment proves that PAK5 gene is vital to cell survival by MTT method. It can be seen from the results that hela cells appear obvious apoptosis after adding staurosporine while hela-PAK5 cells grow well after adding staurosporine. PAK5 plays a role in inhibiting cell apoptosis.

P21-activated kinase5 (PAK5) is the recently identified member of the group II. P21-activated kinase (PAK) family which have been known from its structure, function, mitochondrial positioning and the nucleus shuttle. Although the mechanisms are not completely clear, it plays extremely important role in signal transduction pathways. It provides a strong basis for the study of related disease in apoptosis and opens up a new research direction.

The construction of eukaryotic expression vector of human PAK5 and establishment of stably transfected cell line have laid a good experimental basis for subsequent experiment. We will continue our experiment from mitochondria positioning and signal transduction pathways for a further research.