GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Overexpression, purification, and pharmacologic evaluation of anticancer activity of ribosomal protein L24 from the giant panda (*Ailuropoda melanoleuca*)

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Genet. Mol. Res. 12 (4): 4735-4750 (2013)
Received November 19, 2012
Accepted April 20, 2013
Published October 18, 2013
DOI http://dx.doi.org/10.4238/2013.October.18.11

ABSTRACT. The ribosomal protein L24 (RPL24) belongs to the L24E family of ribosomal proteins and is located in the cytoplasm. The purpose of this study was to investigate the structure and anti-cancer function of RPL24 of the giant panda (*Ailuropoda melanoleuca*). The complementary DNA of *RPL24* was cloned successfully using reverse transcription-polymerase chain reaction technology. We constructed a recombinant expression vector containing *RPL24* complementary DNA and overexpressed it in *Escherichia coli* using pET28a plasmids. The expression product obtained was purified using Ni-chelating affinity chromatography. The results indicated that the length of the fragment cloned is 509 bp, and it contains an open-reading frame of 474 bp encoding 157 amino acids. Primary structure analysis revealed that the molecular weight of the putative RPL24 protein is 17.78 kDa with a theoretical

isoelectric point of 11.86. The *RPL24* gene is readily expressed in *E. coli*, and the RPL24 fused with the N-terminal histidine-tagged protein to give rise to the accumulation of an expected 23.51-kDa polypeptide. The inhibitory rate in mice treated with 0.1 μg/mL RPL24, the highest of 3 doses administered, can reach 67.662%, which may be comparable to the response to mannatide. The histology of organs with tumors showed that the tissues in the RPL24 group displayed a looser arrangement compared with that in the control group. Furthermore, no obvious damage was apparent in other organs, such as heart, lung, and kidney. The data showed that the recombinant RPL24 had time and dose dependency on the cell growth inhibition rate. Human laryngeal carcinoma Hep-2 cells treated with 0.3125-10 μg/mL RPL24 for 24 h displayed significant cell growth inhibition ($P < 0.05$; $N = 6$) in assays using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide compared with that in control (untreated) cells. By contrast, human hepatoma Hep G-2 cells displayed no significant change ($P > 0.05$; $N = 6$) from control (untreated) cells. RPL24 has time and dose dependency on Hep-2 cell growth inhibition. The data indicate that the effect at low concentrations is better than that at high concentrations, and the concentration of 0.625 μg/mL provides the best rate of growth inhibition. Further research is ongoing to determine the bioactive principles of recombinant RPL24 protein that are responsible for its anticancer activity.

Key words: Giant panda; Ribosomal protein L24; cDNA; Cloning; Overexpression; Purification; Anticancer activity