

High expression of human augmenter of liver regeneration in *E. coli*

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Subject **headings** augmenter, liver regeneration; gene expression; DNA; plasmid

INTRODUCTION

Heat-stable hepatocyte stimulatory activity has been described in the liver of weanling rats and pigs. This growth factor is called hepatocyte stimulator substance (HSS)^[1]. Hagiya *et al*^[2] reported the complete amino acid sequence of a 30KDa band from their purified product of rat liver and the cloning and sequence analysis of its cDNA. They called it augmenter of liver regeneration (ALR). Our previous study^[3] demonstrated that human ALR had been cloned and sequenced. To further study the bioactivity of human ALR (hALR), we constructed a highly expressed vector pBV-hALR in *E. coli* and about 20% of somatic protein of rhALR was expressed.

MATERIALS AND METHODS

Materials

Enzymes such as EcoRI, BamHI and Hind III were purchased from Promega. pBV220 plasmid and *E. coli* JM109 were stored and pGEM-hALR plasmid was constructed in our laboratory^[3].

Construction of expressed vector

hALR cDNA fragment was obtained from low-melting gel after electrophoresis of pGEM-hALR plasmid DNA which was digested with EcoRI and BamHI. pBV220 vector DNA was also digested with EcoRI and then incubated at 75°C for 10min. hALR cDNA fragment with compatible cohesive termini and pBV220 DNA fragment were connected in bacteriophage T4 DNA ligation system at 14°C overnight. The reaction contained T4 DNA ligase 1 µL (3u), buffer 10×1 µL, 120 µg hALR cDNA fragment and 100 µg pBV220 DNA fragment. Five µL of ligation mixtures was added to 200µL competent *E. coli* JM109. Transfer appropriate volume of transformed cell onto LB plate agar

containing ampicillin (100 mg/L) at 37°C overnight. Bacterial colonies that contain hALR plasmids by digestion were identified on plasmid DNA with restriction enzymes (EcoRI, BamHI) and agarose gel electrophoresis.

Expression in *E. coli*

LB medium containing ampicillin (100 µg/ml) was inoculated with one colony which contain hALR plasmid to 0. D600 = 0.4 - 0.5 at 30°C and the temperature of the culture was regulated to 42°C, and the incubation was continued for 5 hours.

Separation and purification of granule

It refers to reference^[4].

RESULTS

pBV-hALR expression vector

hALR cDNA fragment was inserted into pBV200 vector (Figure 1) and 6 positive colonies were detected and identified by Hind III. pBV200 vector showed 3 fragments 2727bp, 783bp and 125bp and pBV-hALR vector also showed 3 fragments, 2727 bp, 1183 bp and 125 bp, but the second fragment appeared differently (Figure 2).

Expression in *E. coli*

Induced by temperature, the hALR gene was highly expressed in *E. coli*, and SDS-PAGE showed that 20% of somatic protein of rhALR was expressed (Figure 3). Most of rhALR protein existed as inclusion bodies in *E. coli*. After isolation and washing the purity of granule reached 70% (Figure 3).

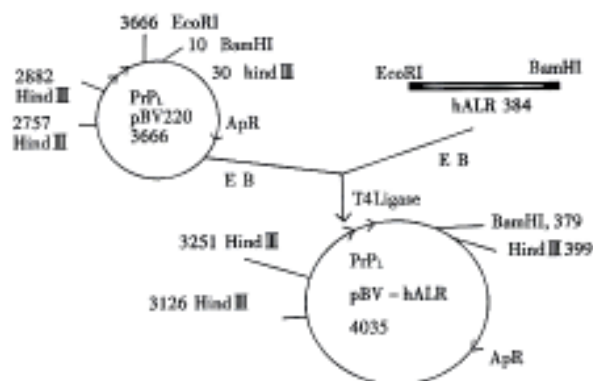


Figure 1 Construction pBV-hALR vector.

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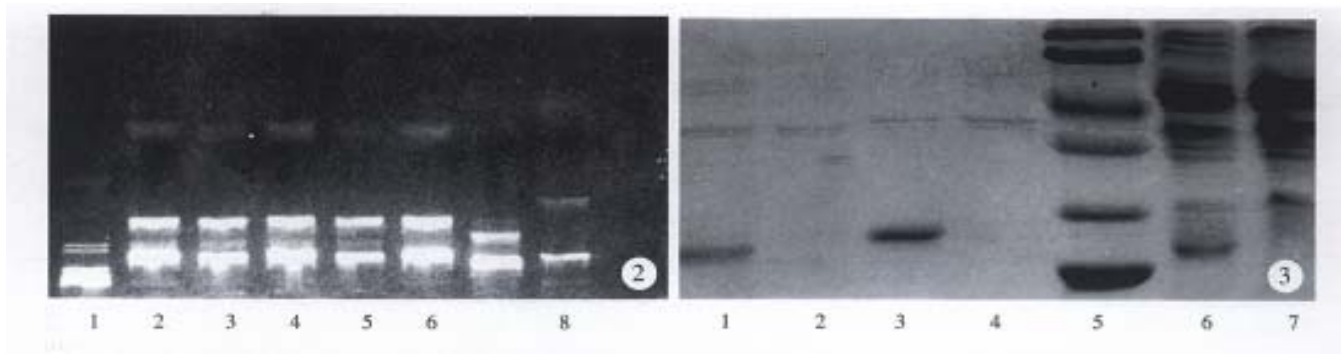


Figure 2 Detection pBV-hALR vector by Hind III.

1 γ /Hind III DNA marker 2-6 pBV-hALR vector digested by Hind III 8 pBV-220 vector digested by Hind III

Figure 3 SDS-PAGE profile of expressed rhALR.

1 Precipitation of *E. coli* JM109 with pBV-hALR after sonication

3 Precipitation of *E. coli* DH5 α with pBV-hALR after sonication

5 Protein standard (Mr 14 400, 21 500, 31 000, 45 000, 66 2000, 97000)

6 *E. coli* JM109 with pBV-hALR

2 Precipitation of *E. coli* JM109 with pBV-220 after sonication

4 Precipitation of *E. coli* DH5 α with pET after sonication

7

E. coli JM109 with pBV-220

DISCUSSION

Since LaBreeque^[1] reported a kind of heat-stable, liver specific stimulator in weanling liver extraction, the gene has been cloned for about 20 years, but in vain. In 1994 Hagiya^[2] found a similar stimulator to HSS (ALR). The recombinant ALR eventually produced by the gene derived from the purified rat cytosol retained the hepatotropic potency as the native peptide, with no effect on the cultured hepatocytes. ALR is not only expressed in liver tissue but also in thymus. With the availability of the hALR gene and its rhALR product we think a series of questions can be explained about its specificity, heat-stable quality and mechanisms. Its

potential clinical implication for the treatment of liver diseases, including fulminant liver failure, is worth further studies.

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