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Construction of eukaryotic expression vector of HBV x gene

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INTRODUCTION

Chronic infection with hepatitis B virus is closely related to liver diseases, including hepatocellular carcinoma. Hepatitis B virus x gene and its product HBx Ag possibly play an important role in carcinogenesis of hepatocellular carci noma. HBV x gene can integrate into cellular DNA during chronic infection[1]. HBx Ag overexpression may alter signal transduction pathways of hepat ocyte[2]. HBx Ag can bind to and inactivate negative growth-regulatory molecules such as tumor suppressor p53[3], suggesting its role in hepa tocarcinogenesis.

We have constructed the HBV x gene eukaryotic expression vector in order to study its contribution to chronic hepatitis and hepatocellular carcinoma.

MATERIALS AND METHODS

Plasmids

Plasmids p^{TTHK} containing HBV DNA and p^{T7Blue} were provided by Dr. ZHANG Huai-Zhong. PCR primers were synthesized by Shanghai Shengon Biology Coporation. Plasmid p$^{CDNA3.1}$ was obtained from our department.

Enzymes and bacterial cells

All restriction enzymes and T4 DNA ligase were purchased from Hua Mei Coporation. *E.coli* JM109 and DH5α were obtained from our department.

Construction of p^{T7Blue}-HBX

Polymerase chain reaction (PCR) was used to obtain HBV x gene from the plasmid p^{TTHK}. The sequence of primers was as follows. The restriction enzyme sites of *EcoR* I and *Kpn* I were added at 5' end of upper and lower primer s respectively.

5'ATCGGTACCATGGCTGCTAGGCTG 3'

5'GGAGAATTCATGATTAGGCAGAGGTG 3'

The condition of PCR is 94℃ 5min, 59℃ 1min, 74℃ 1min, 30 cycles and 74℃ 10min. The PCR results are shown in Figure 1. The PCR product was recovered from the low melting agarose gel and was digested by endonuclease *EcoR* I and *Kpn* I simultaneously and was ligated to plasmid p^{T7Blue} with T4 DNA ligase. A fter recombinant plasmid p$^{T7Blue-HBX}$- was introduced into DH5α, seven ampicillin resistant clones were selected and the plasmid DNA was extracted. The correct plasmids identified by the restriction analysis were sequenced.

Construction of eukaryotic expression vector p$^{CDAN3.1-HBX}$

Plasmid p$^{CDNA3.1}$ and p$^{T7Blue-HBX}$ were digested by endonucl ease *EcoR* I and *Kpn* I. 480bp fragment of HBX and 5.4kb frag ment of p$^{CDNA3.1}$ were recovered from the low melting point agarose gel respectively. They were ligated by T4 DNA and named p$^{CDNA3.1-HBX}$. The reco mbinant DNA was introduced into DH5α. Eight clones were selected and correct plasmids were identified by combinative digestion of *EcoR* I and *Kpn* I. One colony exhibited 480bp and 5.4kb was in right juncti on named p$^{CDAN3.1-HBX}$.

RESULTS

Construction of p$^{T7Blue-HBX}$

In order to construct the eukaryotic expression vector of HBV x gene, we first constructed the vector p$^{T7Blue-HBX}$. The PCR result and the gene structure of recombinant plasmid are shown in Figures 1 and 2. The result of the sequence is shown in Figure 3.

Construction of eukaryotic expression vector p$^{CDNA3.1-HBX}$

On the basis of correct sequence result of p$^{T7Blue-HBX}$, we inser ted HBV x gene at the sites of *EcoRI* and *Kpn* I of p$^{CDNA3.1}$ to construct the eukaryotic expression vector p$^{CDNA3.1-HBX}$. The recombin ant plasmid gene structure is shown in Figure 4.

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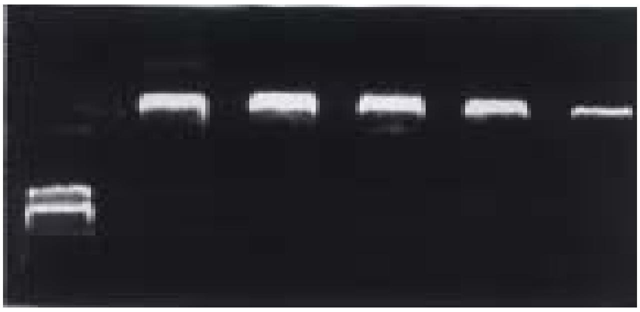

Figure 1 The PCR product of HBV x gene. Lane 1: λDNA/*Hind* IIImarker; Lane 2-6: the PCR product of HBV x gene.

Figure 2 The structure of recombinant plasmid p[T 7Blue-HBX]. Lane 1: λDNA/ *Hind* III marker; Lane 2 - 3: p[T7Blue-HBX] combinative digested by EcoR1 and Kpn1; Lane 4: p[T7Blue] combinative digested by *EcoR* I and *Kpn* I; Lane 5: p[T7Blue-HBX].

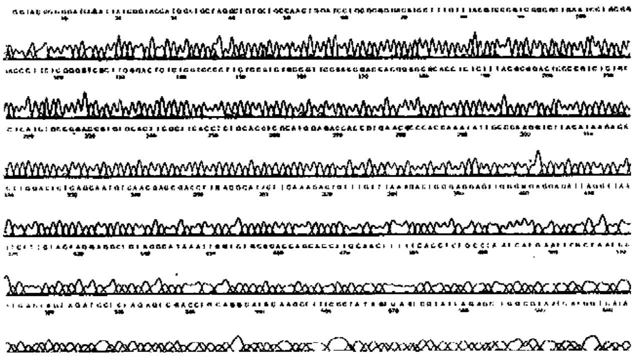

Figure 3 The sequence result of p[T7Blue-HBX].

Figure 4 The structure of p[CDNA3.1-HBX].
Lane 1-2: p[CDNA3.1-HBX] combinative digested by *EcoR* I and *Kpn* I; Lane 3: λDNA/EcoRI marker.

DISCUSSION

Hepatitis B virus (HBV) is a formidable threat to public health. HBV associated HCC is one of the 10 commonly encountered cancers in the world. The relative risk of HBV carriers developing HCC approaches 200:1, which is one of the highest relative risks known for a human cancer[4]. HBV x gene and x antigen may play an important role in the development of chronic infections and chronic liver disease. HBV can integrate into cell DNA and the HBV x region is the most frequently integrated sequence[1]. These integrated fragments make HBV encode x antigen that is capable of trans-activation both *in vitro* and *in vivo*[5]. HBx Ag can stimulate the cell growth directly[6], and inactivate the negative growth regulators, such as tumor suppressor p53[2]. Furthermore, HBx Ag can increase the resistance of HBx Ag-positive cells to apoptosis mediated by cytotoxic cytokine through altering signal transduction pathway of hepatocyte[2].

In conclusion, the topic of HBV is an old but important one, and is worthy of further studies. We have constructed the eukaryotic expression vector of HBV x gene for this study.

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