Construction of retroviral vector carrying HSV-tk gene under control of human AFP enhancer core sequence and human pgk promoter

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AIM: To construct retroviral vector bringing HSV-tk gene under control of human AFP enhancer core sequence and human pgk promoter.

METHODS: Internal SV40 promoter was deleted by SalI from retroviral vector pMNNM to construct pMNAP. HSV-tk gene driven by pgk promoter was released by BamHI from an eukaryotic expression vector pBPGK-tk, and inserted into polylinker site of pMNAP to construct pMNP-tk retroviral vector. Human α-fetoprotein gene enhancer core sequence was released by EcoRI from pGEM-7Z-AFPf plasmid was inserted into the immediate upstream of pgk promoter of pMNP-tk vector. Construction of hepatoma specific retroviral vector pMNAP-tk was completed.

RESULTS: The structure of pMNP-tk and pMNAP-tk vector was confirmed by restriction analysis.

CONCLUSION: The vector is of great significance for hepatoma specific prodrug transformation gene therapy.

Key words: Liver neoplasms; Herpes; Simplex virus; Retroviral; Alpha fetoproteins; Enhancer elements; Gene therapy

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COMMENTARY

Gene therapy for tumor is a modern biotherapy which is rapidly developing at present. The key point of gene therapy is to introduce the gene of interest into the tumor cells, and make the genes specifically express in tumor cells. Tumor targeted expression of genes was fully emphasized, especially to employ the cell type specific expression regulatory elements to direct the expression of the genes in certain target cells. American Recombinant DNA Advisory Committee (RAC) of NIH had passed a protocol which was suggested by K.W. Culver. That was to transfer HSV-tk gene into the murine packaging cells and introduce these cells into the human brain tumor bed, and followed by ganciclovir (GCV) treatment. GCV, by conversion into GCV triphosphate, can inhibit DNA polymerase, resulting in inability of the cells to proliferate. Now it has been brought into clinical experiments. Gao et al in this study improved this method, by constructing retroviral vector PMNAP-tk to the upstream of HSV-tk gene human pgk promoter using human AFP enhancer core sequence. This vector has the function of hepatoma specific prodrug transformation. This study on hepatoma gene therapy should be of great significance. Professor Zu-Yu LUO, Institute of Life Science, Fudan University, Shanghai, China.

INTRODUCTION

Herpes simplex virus thymidine kinase (HSV-tk) can catalyze deoxythymidine to both deoxythymidylic acid and some nucleoside analogues (NAS) phosphorylation. These phosphorylated NAS is more toxic to mammalian cells, blocking cell DNA duplication[1]. Recently, numerous experiments in vivo or in vitro showed that HSV-tk gene transferred into tumor cells by some shuttle vectors produces efficiently anti-tumor effect using NAS as prodrug[2-5]. Furthermore, the clinical trials have been approved in some countries for the treatment of brain tumor with HSV-tk gene/prodrug system[6]. Morbidity of hepatic cancer is very high in China. No effective treatment is available for the tumors in late stage. In order to establish an effective gene therapy against hepatic cancer and study the effect of household gene enhancer in the retroviral shuttle vector to regulate the gene of interest for hepatoma specific expression, we have constructed the general and the hepatoma specific retroviral vector.
specific HSV-tk retroviral expression vector.

MATERIALS AND METHODS

Plasmids

Retroviral shuttle vector pMNSM was provided by Tokyo Medical and Dental University. Plasmid pBGK-tk containing the human phosphoglycerokinase gene (pgk) promoter and HSV-tk gene was obtained from Nara Medical University. Plasmid pGEM 7Z-AFP containing a human α-fetoprotein gene enhancer core sequence was provided by T. Tamaoki (University of Calgary, Calgary, Canada).

Enzymes and bacterial cells

All restriction enzyme, T4 DNA ligase and Klenow fragment were purchased from Promega Corporation. E. coli host strain HB101 and E. coli JM109 were obtained from our department. The general and hepatoma specific HSV-tk retroviral expression vectors were constructed[1]. After plasmid pMNSM and pBPGK-tk were introduced into E.coli HB101, respectively, ten ampicillin resistant colonies were selected and the plasmid DNAs were extracted. The correct plasmids identified by the restriction analysis were amplified through 30 cycles of PCR and purified by PEG 8000 (Sigma Corporation) method, and were digested by EcoA, HindIII, BamHI and HpaI. The digested plasmid pMNSM was digested by endonuclease SalI, and the small SV40 promoter fragments were discarded and the big fragments were recovered from the low melting point agarose gel, and inserted into the SalI linearized plasmid pMNP-tk. The recombinant DNA was introduced into the E.coli HB101 and combinative digestion of HindIII and BamHI were used to identify the correct colonies. One colony exhibiting 406 bp, 2.8 kb and 6.4 kb fragments was in right junction, named pMNP-tk α. Another colony exhibiting 321 bp, 2.8 kb and 6.5 kb fragments was reverse, named pMNP-tk β (Figure 2).

RESULTS

Construction of pMNP-tk

In order to make the chimeric HSV-tk gene express in eukaryotic cells, we isolated the DNA fragments containing both the pgk gene promoter and HSV-tk gene from the eukaryotic expression vector pBGK-tk and removed the SV40 promoter from the retroviral vector pMNSM. The recombinant plasmid gene structure is shown in Figure 3.

Construction of hepatoma specific expression retroviral shuttle vector[8]

Plasmid pGEM 7Z-AFP DNA was transformed into E.coli JM109. By α complementation method, the white color colonies were selected and the correct plasmids were identified by the restriction analysis (Figure 1). The credible plasmid pGEM-7Z-AFP was amplified. The AFP gene enhancer core sequence 727 bp was further amplified, and was being filled in by Klenow fragment and dNTP, then self-circularized with T4 DNA ligase, and inserted into the SalI linearized plasmid pMNP-tk. The recombinant DNA was introduced into the E.coli HB101 and combinative digestion of HindIII and BamHI were used to identify the correct colonies. One colony exhibiting 406 bp, 2.8 kb and 6.4 kb fragments was in right junction, named pMNP-tk α. Another colony exhibiting 321 bp, 2.8 kb and 6.5 kb fragments was reverse, named pMNP-tk β (Figure 2).

DISCUSSION

The gene therapy for cancer is developing rapidly. Its mechanisms may be as follows: Increasing the anti-tumor immunity, and introducing tumor inhibitor gene, the antisense of oncogenes, the prodrug converting gene, MDR-1 gene for the protection of chemotherapy, and the anti-metastasis gene[23]. The prodrug genes, such as HSV-tk, VZV-tk and cytosine deaminase (CD), and encode proteins, can convert the nontoxic prodrug into intracellular toxins as non-mammarian metabolic enzymes[13]. Such enzymes can block the cell DNA duplication as a competitive inhibitor of DNA polymerase.

The gene expression in tumor cells is the first step for gene therapy. Eukaryotic expression plasmid vector and viral vector are commonly used for the expression of genes in tumor cells. In viral vectors, the recombinated retroviral vector, which is constructed with MoMuv as the main skeleton, is more often used[20]. It is feasible that a gene will be expressed if it is regulated by two promoters. In our construction, we deleted the SV40 promoter of the retroviral vector, and made the tk gene under the control of the pgk gene promoter, which is a kind of eukaryotic promoters. These may decrease the presence of wild retrovirus[22] caused by recombinated homologous product.

In vivo gene therapy, it is important to make the gene specifically express in tumor tissues. Two approaches may be used.
One is to modify the shuttle vector by gene engineering, the other is to direct the gene expression in target cells by means of the tissue specific transcriptional regulatory sequence (TRS). Scharfmann et al[9] considered that the transcriptional regulatory sequence of "household gene" will cause the chimeric gene to express at a high level in a specific tissue. Hubber et al[7] reported that VZV-tk gene regulated by AFP TRS and mediated by retroviral vector, made the AFP-positive hepatoma cells, HepG2, H3B and HuH7 sensitive to the prodrug 6 methoxypyrimidine arabinonucleoside (araM). The aim of our work was to regulate the pgk + tk gene by AFP enhancer core sequence 727 bp. The usage of the TRS for regulation of HSV tk gene can not only increase its transcription but also make its expression tissue specific. In our future experiments, we will assess their anti-tumor effects in nude mice.

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