IMAGE OF HSV1-TK GENE EXPRESSION WITH ¹²³IVDU

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1. Introduction

The liver is an important target organ for gene transfer due to its capacity for synthesizing serum protein and its involvement in numerous genetic diseases. So livertargeted gene transfer is significant tool for expanding the treatment options and gene function studies.

Gene transfer methods commonly use recombinant viral vector. However, viral vectors also have various disadvantages for example immune recognition after adenoviral vector delivery and potential viralassociated toxicity including helper virus replication and insertional mutagenesis.

In contrast, nonviral vectors such as naked plasmid DNA(pDNA) and cationic liposomal systems exhibit low immunogenicity and repeated administration is possible(Ledley et al.,1992; Nabel et al.,1993) These are attractive vectors for in vivo gene transfer because of their suitable characteristics such as biodegradability, minimal toxicity, nonimmunogenicity, and simplicity of use. But non-viral gene delivery, has problems associated with limited efficiency at gene expression.

hydrodynamic-based produce has very high level efficiency of gene extraction in liver or soild tumor. In mice, hydrodynamic-based produce was reported that a high level of transgene expression could be obtained in the liver by intravenous injection of largevolume(8~10% of body weight) and high-speed (Kobayashi N et al., 2004).

HSV1-TK is one of the most widely use effecter gene systems sued for imaging gene expression, in association with its use as a suicide gene, or as a reporter gene In non-invasive imaging of the HSV1-TK system, many nucleoside derivatives have developed as prodrug for tumor proliferation imaging or as anti-viral drugs. Several 5-substituted uracil nucleoside derivatives have been identified to have high sensitivity and selective accumulation in HSV1-TK expression cell. This producer has been used hydrodynamic-based produce, we investigated to image of herpes simplex virus type 1 thymidine kinase (HSV1-tk) gene with (E)-5-(2-Iodovinyl)-2⁻-deoxyuridine (IVDU) as HSV1-tk reporter probe.

2. Methods and Results

2.1 recombinant plasmid DNA

Enhanced fluorescent green protein plasmid(pEGFP/N1) was purchased from BD biosciences clontech. pEGFP-TK/N1 was constructed by insertion of Nco I/EcoR V HSV1-tk cDNA as of pMOD into pEGFP/N1 and than TK/N1 was completed by EGFP cDNA was removed from pEGFP-TK/N1 (figure.1). Each pDNA was amplified in the DH5 α stain of Escherichia coli and purified using a QIAGEN high-speed plasmid midi kit (Qiagen GmbH, Hilden, Germany). We used the pEGFP/N1 as a control and pTK/N1 for an investigation.



Figure1. Recombinant plasmid DNA

In first of experiment, we constructed pTK/NI. pEGFP-TK/N1 was constructed by insertion of Nco I/EcoR V HSV1-tk cDNA as of pMOD into pEGFP/N1. TK/N1 was completed by EGFP encoding gene was removed from pEGFP-TK/N1.(Figure 1A).

2.2 RT-PCR at McA RH-7777

Recombinant pTK/NI was tested by RT-PCR On the 1day after the transfection at McA RH-7777 cell, . Total RNA was extracted from McA RH-7777 using RNeasy mini kit (Qiagen GmbH, Hilden, Germany). RT-PCR was used Onestep RT-PCR kit (Oiagen GmbH, Hilden, Germany) with a HSV1-tk specific primer and 200ng of total RNA as template. The HSV1-tk specific forward primer 5'was CTCACCCTCATCTTCGACCG-3end, and the reverse primer was 5'-CCTGCAGATACCGCACCGTA-3end. This set of primers were designed by amplifies a 290bp segment of HSV1-tk coding sequence. RT-RCR was using the following profile : (A) 95°C for 15min (B)

95°C, 60°C and 72°C for 1min ; total 35 cycle (C) 72°C for 10min. We confirmed the PCR products by 1.5% agarose gel electrophoresis.(figure. 2)

M McA McA RH-7777 RH-7777(TK expression)



Figure 2. TK gene Expression in McA RH-7777cell detected by using PCR. Gene expression in McA RH-7777cell at 1 days later(Figure 2). as shown Figure 2, transfection of pTK/NI was determined high level tk gene expression and transfection of pEGFP/NI was not determined tk gene expression by RT-PCR at used tk specific primer.

2.3 Biodistribution

Mice's tail vein in the anesthetized condition by ether were injected with 40ug of pTK/N1 or pEGFP/N1 used hydrodynamic-produce. 1day post-injection, in-group of four mice each, carrier-added [¹²³I] IVDU was administered into the mice's tail vein. 4hour later, the mice were sacrificed, and the organ of interest and blood were collected, weighed, and radioactivity-using gamma counter. The radioactivity in each organ was expressed as percentage of the injected does. As shown Figure 3, HSV1-TK was high expression in liver.



Figure. 3 TK gene Expression in organ detected by using Gamma counter.

2.4 Gamma Camera image

mice's tail vein in the anesthetized condition by ether were injected with 40ug of pTK/N1 or pEGFP/N1 used hydrodynamic-produce. After 1day and 3day, [¹²³I] IVDU (50uci/100ul) injected by mice's tail vein. Immediately 2hours later, the mice were imaged by gamma camera.

In gamma camera images, ¹²³IVDU uptake was selectively localized at 1 day and at 3 day, and 1day



Figure. 4 TK gene Expression in organ detected by using Gamma camera.

uptake of liver of pTK/N1 injected mice was more accumulated 3 day uptake in liver of pTK/N1 injected mice. ¹²³IVDU showed minimal uptake in liver of pEGFP/N1 injected mice.

3. Conclusions

¹²³IVDU uptake in liver of pTK/N1 injected mice was higher than that of pEGFP/N1 injected mice and was selectively localized in gamma camera image. These results suggest that ¹²³IVDU could be used for the expression of pTK/N1 in gamma camera image.

4. Reference

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