

Protein transduction domain of membrane penetrating peptide can efficiently deliver DNA and protein into mouse liver for gene therapy

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BACKGROUND: The development of a harmless and efficient nonviral gene delivery system that can facilitate the penetration of nucleic acids through the plasma membrane is a key to successful gene therapy. The aim of this study was to test a nonviral gene transferring vector's function of delivering DNA into liver cells to provide an important clue for gene transfer in liver gene therapy.

METHODS: The complex of DNA and DNA delivering protein was injected into mice through their tail veins. Then the mice were killed and their liver tissue was sectioned. The gene transferring results were detected using a confocal laser scanning microscope.

RESULTS: Fluorescence analysis indicated that both DNA-membrane penetrating peptide (MPP) complex and DNA-hepatocyte specific receptor binding domain (HSRBD)-MPP complex could go into liver cells. The fluorescence value of liver cells in the DNA-HSRBD-MPP group was higher than that in the DNA-MPP group.

CONCLUSIONS: MPP can successfully deliver DNA and protein into cells, and MPP with a HSRBD can specifically deliver DNA into liver cells. These have laid a foundation for further study on the nonviral liver cell gene delivering system.

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KEY WORDS: membrane penetrating peptide;
gene therapy;
gene delivering

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Introduction

Recent progress in basic and clinical biomedical research has been largely dependent on the development of gene delivery technologies, including recombinant viruses (viral vectors) and other delivery strategies (nonviral vectors). Nevertheless, the development of a novel and efficient nonviral delivery system is an important goal, because recombinant viruses still have a number of disadvantages as practical tools for medical application.^[1] Recently, the roles of various chemical and biological agents have been examined in the facilitation of gene transfer. These agents include endosmotropic agents, inactivated adenovirus particles, synthetic and natural amphiphilic peptides, cationic polymers, and synthetic neutral phospholipids.^[2] The utility of these agents, however, is largely circumscribed, especially for *in vivo* application, by high toxicity and the instability of the complexes in the presence of serum proteins.^[2] Therefore, the development of a novel and harmless agent that can facilitate the penetration of nucleic acids through the plasma membrane is a key to successful DNA delivery.

Recently, some transcription factors, including the Tat protein of human immunodeficiency virus,^[3,4] VP22 protein of herpes simplex virus,^[5] and antennapedia protein of *Drosophila*,^[6] have been shown to penetrate the plasma membrane. The peptide segments responsible for membrane penetration, the protein transduction domain (PTD), consisting of 11-34 amino acid residues, have no uncommon feature, except the presence of basic amino acid residues (arginine and lysine), which may be involved in contact with the negatively charged lipids or in membrane penetration.^[7-9]

In this study, we examined the potential of the PTD of MPP protein as an agent to stimulate gene transfer. We designed a novel peptide with a high transduction domain, enhanced green fluorescence protein (EGFP), and Poly-His section, in which, there is a hepatocyte specific receptor binding domain (HSRBD) to specify mediation to the liver. We used HSRBP and some DNA-protein complex which binds MPP, to test their

penetrating ability in mice liver cells.

Methods

Mouse body internalization of DNA-MPP complex and DNA-HSRBD-MPP complex

Four mice of Kunming strain weighing 15–20 g were used in this experiment and they were randomly divided into 2 groups. One group was given MPP-DNA complex with HSRBD and another non-HSRBD. The complex [0.1 ml (0.93 mg/ml)] was injected into the tail vein of the mice for 2 times with an interval of 10 minutes. The control group was given EGFP peptides without MPP.

Preparation of frozen tissue sections

Tissue samples from surgical specimens of the liver were dipped in 0.9% NaCl, snap frozen in liquid nitrogen for 2–5 minutes immediately after removal, and then transferred to a cryostat at -20 °C. Serial frozen sections were cut at 10 μm, air-dried for 30 seconds and washed in PBS for 2 times. The sections were fixed in 80% acetone at room temperature for 10 minutes, air-dried for 15 minutes at room temperature, and then subjected to staining. The samples were incubated with 0.5% TritonX-100 (PBS) for 5 minutes, and then treated with 3% H₂O₂ (PBS) for 15 minutes. The first antibody was

incubated at 37 °C for 1.5 hours (first antibody: anti-GFP, 1:200–1:400 by PBS), and then washed with PBS for 5 times, 3 minutes each time. The secondary antibody (Texan red labeled sheep anti rabbit IgG) was incubated for 30 minutes, and washed with PBS for 5 times, 3 minutes each time. The data were obtained with a confocal laser scanning microscope demonstrating the green fluorescence at 488 nm and 620 nm.

Fluorescence and microscopic analysis

Transfected cells were examined directly with an Olympus microscope. Confocal images were taken under a Zeiss confocal laser scan microscope. Cells would grow on the cover lip after peptides transfer. One hour after the transfer, the cover lip was put on the carry plate and the green fluorescence was observed at 488 nm.

Results

Internalization into mouse liver with HSRBD (Fig. 1)

In this experiment, 0.1 ml (0.137 mg/ml) preparation was injected into the mouse through the tail vein to observe the delivery of HSRBD into the liver of the mouse. After 30 minutes, we observed MPP with HSRBD was delivered into mouse liver, whereas MPP without HSRBD showed negative result (Fig. 2).

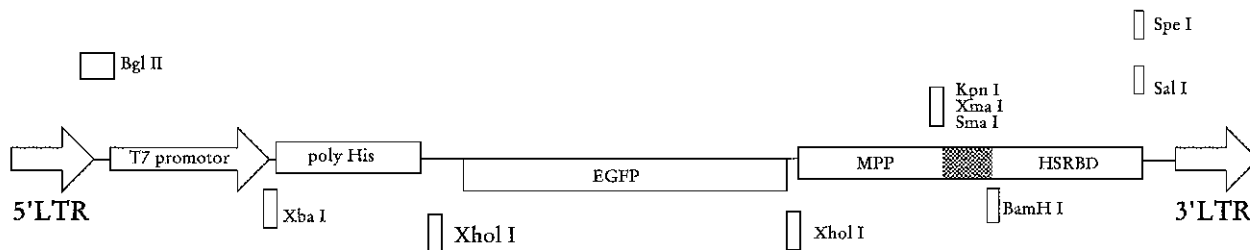


Fig. 1. Nonviral HSRBD vector specific for gene transfer into the liver.

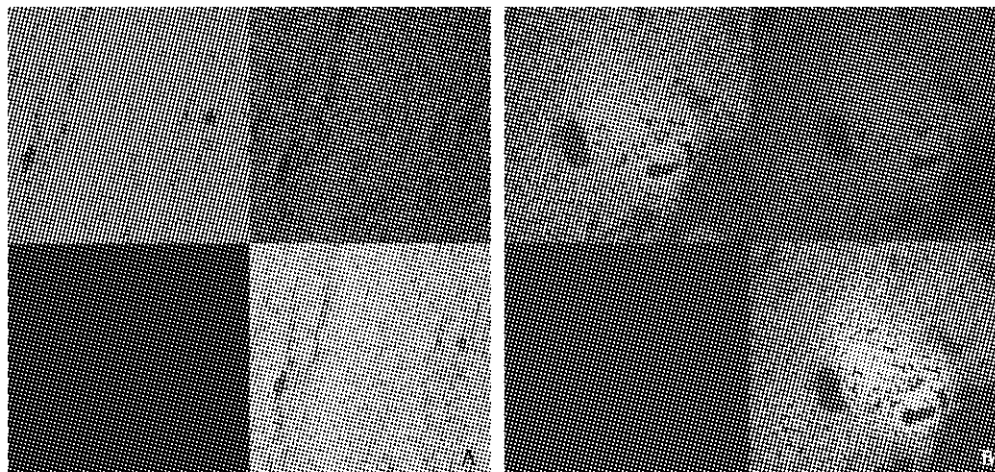


Fig. 2. Fluorescence photos showing mouse liver after HSRBD delivery (A: internalization with MPP; B: internalization with HSRBD).

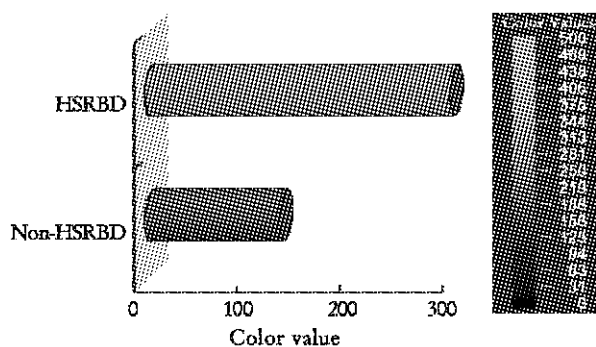


Fig. 3. Comparison of the fluorescence of HSRBD and non-HSRBD peptides

Fluorescence of HSRBD and non-HSRBD peptides

The values of internalized HSRBD and non-HSRBD peptides were analysed (Fig. 3).

The values of fluorescence in the mouse liver stimulated by HSRBD were the same as those by non-HSRBD. The lightness of fluorescence shown by the two ways was not significantly different suggesting that the specific domain, which binds the apo-E receptor in the liver cell, does not affect the internalization of MPP-mediated peptides and that HSRBD, which specifically binds the apo-E receptor domain, has the same delivering function as the non-HSRBD peptides.

Discussion

In recent years, several peptides have been demonstrated to penetrate into the plasma membrane of eukaryotic cells via a seemingly direct pathway.^[4,10,11] These peptides have been successfully used in the intracellular delivery of macromolecules with several advantages over conventional techniques because they are efficient for a range of cell types, can be applied to cells thoroughly, and have a potential application of treatment.^[5,12] The current knowledge of membrane-penetrating peptides has suggested that many additional sequences could be designed and applied for the cellular delivery of drugs or as research tools.

Recent researches in MPP and its family members have shown that MPP might be effective in cargo function to deliver macromolecules into cells.^[13-19] In our study, we found that MPP and the MPP-vector-DNA complex were internalized into the liver of mouse 30 minutes after their injection through the tail vein.

The therapeutic potentiality of transducing proteins has been demonstrated in tissue culture studies.^[20-22] To determine whether a large biologically active protein could be successfully transduced *in vivo*, we used the 30 kD EGFP protein. An C-terminal MPP-EGFP fusion protein was generated, EGFP fusion protein missing the MPP PTD but retaining the rest of the sequence was set

as a control. To supply the specific binding to hepatocyte cells, an apo-E derived peptide was linked to the C-terminal of the novel vector.

With the development of many new techniques, the technology of gene transfer has been matured rapidly, but the difficult though important task is to deliver hydrophilic macromolecules across the blood-brain barrier. Several methods have been envisaged to overcome this hurdle; but they have limitations such as effectiveness being restricted to a subset of molecules or low yield in brain delivery. In our experiment, however, MPP mediated EGFP protein inspiring green fluorescence suggested that membrane-penetrating peptides might be able to transport macromolecules through the blood-brain barrier.

In conclusion, a specific vector specifically binding to liver tissue was constructed based on an apo-E derived peptide that binds the apo-E receptor on the surface of the liver. This method may be helpful in the development of specific gene therapies for specific tissues.

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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The best physician, one fit to treat a king, is he whose knowledge is fourfold: the cause, symptom, cure, and non-recurrence of disease.

The Carakaa Samhita (ancient Indian text)