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Received 5 February 2003; accepted 6 April 2004.

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In utero detection of T7 phage after systemic administration to pregnant mice

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BioTechniques 37:81-83 (July 2004)

The phage is used as a scaffold to display recombinant libraries of peptides, which provides the means to rescue and amplify peptides that bind target macromolecules. Many reports showed that the T7 phage display method can be used to obtain a ligand-binding peptide for tissue-targeted therapies in adult animals. In utero tissue targeting of fetal tissues may help in the correction of many genetic and metabolic diseases. Here we demonstrate the distribution and detection of T7 phage displaying the C-X7-C peptide library in mouse fetal tissues after systemic injection of T7 phage into pregnant mouse tail vein. T7 phage was recovered from fetal tissues 15 min after injection of T7 phage. Our results suggest that T7 phage may be a useful tool in selecting the tissue-specific ligand-binding peptide for fetal tissues. This approach may be helpful in designing in utero tissue-targeted therapies.

INTRODUCTION

Prenatal in utero gene transfer may eventually become a useful approach for the correction of various genetic disorders. This method of gene transfer may prove to be advantageous in rapidly replicating fetal cells and may also be less likely to induce a host immune response to a vector or transgene product due to the less well-developed state of the fetal immune system. The phage display method of combinatorial chemistry has become a very popular means of searching ligands with high affinities to a given target (1–3). Phage display is a powerful method for selecting and engineering polypeptides with the desired binding specificities (4,5), and phage display peptide libraries are commonly used to obtain defined peptide sequences interacting with a specific molecule. In this system, peptides in as many as 10^9 permutations are expressed on the phage surface by fusion to one of the phage surface proteins, and the desired peptides are selected on the basis of binding to the target molecule (6). In vivo phage display is a powerful method to identify organ- and tissue-specific

vascular addresses. This approach consists of intravenously injecting a phage library into a mouse, allowing the phage to circulate in vivo, and then collecting the phage bound to a specific organ or tissue of interest. The strength of this technology is its ability to identify interactive regions of proteins and other molecules without preexisting notions about the nature of the interaction. Phage libraries have been reported for the selection of peptides that bind immobilized proteins (4,5,7,8) and carbohydrates (9) and also for peptides that bind to cultured cells and tissue vasculature (1,6). These reports deal with the targeting of adult tissues using the phage display system. No report is available for targeting fetal tissues by the phage display system using the maternal systemic circulation. Here we report the distribution of T7 phage displaying the C-X7-C (C, cysteine; X, any amino acid) peptide library in fetal tissues after injecting it into the systemic circulation of a pregnant mouse.

MATERIALS AND METHODS

Phage Peptide Libraries, Amplification, and Purification

T7 phage (T7Select415) was obtained from Novagen (Madison, WI, USA). This system has the capacity to display peptides up to about 50 amino acids in size in high copy number (415 per phage). T7 phage displaying the C-X7-C (C, cysteine; X, any amino acid) peptide library was made according to Rajotte et al. (1) and Novagen. Titer of the phage was approximately 10^{12} plaque-forming units (pfu)/mL. In brief, the phage was amplified in a log phase of 0.5 L BL21 *Escherichia coli* strain at 37°C for 3 h. After removal of cell debris by centrifugation at 11,900× *g* for 10 min, the phage was precipitated with 10% polyethylene glycol (MW 8000) and extracted from the pellet with 1 M NaCl, 10 mM Tris-HCL, pH 8.1, 1 mM EDTA, followed by a cesium chloride gradient ultracentrifugation at 210,000× *g* for 60 min. Carefully collected T7 phage bands were subjected to dialysis against phosphate-buffered saline (PBS) for 1 h at 4°C and stored at 4°C for future use.

T7 Phage Distribution in the Fetal Tissues

Adult female Balb/C mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and allowed to mate at the University of California San Diego animal facility (La Jolla, CA, USA). Embryonic day (E)14 pregnant mice were used for the experiments. Mice were first anesthesized with isoflurane and injected in groups of three for each experiment. Experimental groups were injected with 10^9 pfu T7 phage particles, and control groups were injected with PBS, both using the tail vein. Fifteen minutes after injection, each mouse was perfused with 20 mL PBS followed by 10 mL 10% formalin through the aorta. The perfusion was released through a puncture in a left heart ventricle. The fetuses were collected and fixed in 10% formalin and subjected to a wax block for immunohistochemistry studies. Fetuses from another group of injected mice were dissected, and the tissues (liver, brain and lung, and heart and gut) were subjected to the pfu assay for quantitative determination of distribution of T7 phage in the fetal tissues. The tissue lysates from control fetuses were plated simultaneously to check for T7 phage contamination. Each experiment was repeated in four separate fetuses. In brief, the fetal tissues were homogenized (10% w/v) in LBM9 salt, and the homogenate was incubated with 500 µL of log-phase BL21 *E. coli* cells for 2 min, mixed with 3 mL 0.6% agar in LB medium, and plated onto 1.5% agar in 10-cm plates. The plaques were allowed to develop overnight at room temperature and counted for the presence of the live T7 phage per fetal tissues (1).

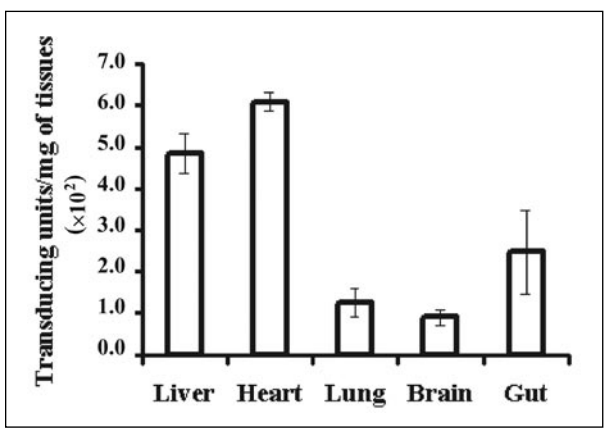

Figure 1. In utero recovery of active T7 phage from different fetal tissues after 15 min of intravenous tail vein injection to the pregnant mouse. T7 phage particles (10^9 pfu) were injected per pregnant mouse. Error bars show the mean from four fetal tissues (± SD). T7 phage was not detected in phosphate-buffered saline (PBS)-injected (control) mice. pfu, plaque-forming units.

Immunohistochemistry

T7 Phage were detected in the fetal tissues by immunostaining as described (8). Briefly, anesthesized experimental mice were injected with 10^9 pfu T7 phage particles. Control mice were injected with PBS only. Both the groups of mice were perfused as described previously (10). Fetuses from pregnant mice were surgically removed and then fixed in 10% formalin for 24 h. All the fetuses were then subjected to immunohistochemistry. The following three controls were always used to ensure the specificity of immunostaining: (*i*) anti-rabbit immunoglobulin G (IgG) isotype matched; (*ii*) rabbit negative serum control; and (*iii*) with anti-vWf (factor VIII, Dako, CA, USA). An antibody against T7 phage (rabbit-anti T7 phage antibody obtained from E. Ruoslahti, The Burnham Institute, La Jolla, CA) was used as the primary antibody (1:1000 dilution) for the staining followed by a secondary horseradish peroxidase (HRP)-conjugated polyclonal antibody (DakoCytomation, Carpinteria, CA, USA). Both the antibodies used were 200 µL/section with a 30-min incubation time at room temperature. The final development of histological sections was completed with 3,3-diaminobenzidine (DAB) without the use of antigen retrieval.

RESULTS AND DISCUSSION

T7 phage was injected into E14 pregnant mice to examine the phage distribution into fetal tissues through the maternal systemic circulation. Fifteen minutes after injection of T7 phage, we perfused the mouse and then subjected them to a pfu

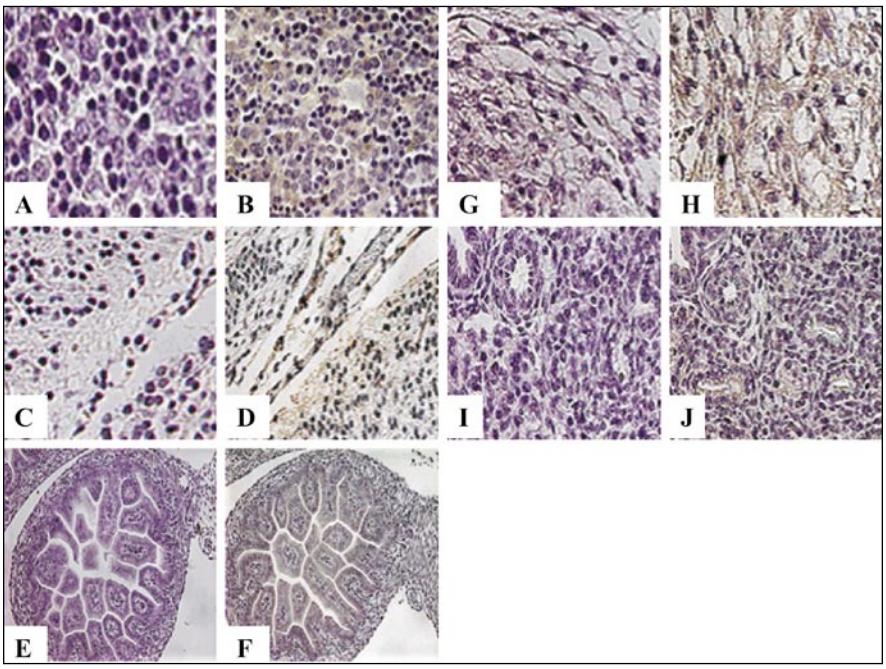

Figure 2. Immunohistochemical staining of fetal tissues. Control mice were injected with phosphate-buffered saline (PBS), and experimental mice were injected with T7 phage using intravenous tail vein into E14 pregnant mice. After 10 min of injection, each mouse was perfused with 20 mL PBS followed by the 10 mL 10% formalin through the aorta. The perfusion was released through a puncture in a left heart ventricle. The fetuses were collected and fixed in the 10% formalin and subjected to a wax block. Panels A, C, E, G, and I show the control fetal liver, brain, gut, heart and lung, respectively, whereas panels B, D, F, H, and J show the immunohistochemical staining of T7 phage-injected fetal liver, brain, gut, heart, and lung, respectively.

assay for determining the presence of live T7 phage in fetal tissues (Figure 1). The highest titer was obtained in heart tissue, whereas the lowest titer was in brain tissue. Whole fetuses were subjected to immunostaining for the distribution of T7 phage on the fetal tissues (Figure 2). Immunohistochemistry revealed the presence of T7 phage in fetal liver, heart, brain, gut, and lung tissues of injected animals, but not in non-injected control animal. Furthermore, liver and heart tissues show homogenous T7 phage distribution, which is exhibited by strong T7 phage antibody staining, in comparison to other tissues studied. The liver and heart tissues are functional in an E14 fetus, and therefore it may be attributable to the increase in blood flow to these tissues that results in an increased amount of phage uptake by these tissues. The alveolar capillaries of the lung were stained, whereas the bronchiolar walls were negative, suggesting that T7 phage displaying the C-X7-C peptide library was unable to bind to large blood vessels and the bronchiolar wall (1). The C-X7-C display peptide did not influence the distribution of T7 phage in mouse tissues. Srivastava et al. have also discussed the in vivo fate of the T7 phage in the adult mouse (10). T7 phage in the mouse was rapidly cleared by the mother's immune system, but enough phage particles were able to infect the fetuses' as well as the mother's tissues (10).

Our data suggests that the T7 phage can cross the placental barrier and may reach up to the fetal tissues. Therefore, it is possible to target the fetal tissues through maternal blood circulation using T7 phage display library.

ACKNOWLEDGMENTS

We thank Theodore Friedmann, (Department of Molecular Genetics, University of California San Diego, La Jolla) for providing the T7 phage, laboratory space, and useful discussions, Rangnath Mishra, [Case Western Reserve University (CWRU), Cleveland, OH] for critical suggestions, Robin Neulin (The Burnham Institute, La Jolla, CA) for immunostaining, and Melinda Richards for excellent administrative support. This work was supported by Ronald McDonald House Charity Medical Grant.

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Received 17 October 2003; accepted 12 April 2004.

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