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Targeting the Choroid Plexus-CSF-Brain Nexus Using Peptides Identified by Phage Display

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Abstract

Drug delivery to the central nervous system requires the use of specific portals to enable drug entry into the brain and, as such, there is a growing need to identify processes that can enable drug transfer across both blood-brain and blood–cerebrospinal fluid barriers. Phage display is a powerful combinatorial technique that identifies specific peptides that can confer new activities to inactive particles. Identification of these peptides is directly dependent on the specific screening strategies used for their selection and retrieval. This chapter describes three selection strategies, which can be used to identify peptides that target the choroid plexus (CP) directly or for drug translocation across the CP and into cerebrospinal fluid.

Keywords

Blood-brain barrier; Choroid plexus; Choroid epithelial cells; CNS targeting; Library screening; Combinatorial biology; Blood-CSF barrier; Drug translocation; *Ex vivo* biopanning; Ligand internalization; Phage display; Screening

1. Introduction

Filamentous bacteriophage are a group of viruses that contain circular, single stranded DNA encased in a long (1 μm) protein capsid. By electron microscopy, they appear as cylinders and, at the very tip of the Ff class of phage (e.g., M13), there are five copies of the plll protein that is encoded by gene lll. Normally, this protein is used by the virus to target and enter *Escherichia coli* that contains the F plasmid and expresses a receptor for this phage. Once inside the bacteria, these phage replicate and are released by the bacteria at a very high titer (10^{11} pfu/mL).

In 1985, Smith (1) conceived of a method whereby short nucleic acid sequences of DNA inserted into the glll gene generate phage that display a peptide-plll fusion protein but retain infectivity for *E. coli*. Reasoning that the displayed peptides could confer phage with new intrinsic activity, he proposed that it should be possible to introduce random sequences of DNA into the glll gene and create complex libraries of peptides. Because each peptide is displayed in five copies on an individual phage, mixtures of these phage, called libraries, can be screened and individual phage particles harvested to select novel peptides that confer novel activities to these phage. They are then enriched by a process called "biopanning" and

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any phage carrying the screened activity is recovered and replicated in bacteria. Sequencing the gene lll of these phage reveals the identity of the peptide that confers the new biological activity to the phage. In a library containing 10^9 different peptide sequences, there is a statistically significant probability that there are 100 different candidate peptides that could be recovered from the library even if the probability of its existence is so low (e.g., $1:10^7$) as to be unrealistic.

Over the past several years, we and other investigators have been adapting the original phage display technique to identify various peptides with different specificities and activities (2, 3). For example, peptides have been identified that induce physical stability of particles in organic solvents like chloroform, decrease complement activation of macromolecules in blood, modify immunogenicity, alter viral tropism *in vitro* and *in vivo*, internalize particles into cells, transduce cells, promote transcytosis *in vitro* and *in vivo*, and even promote transmigration of particles across cell barriers *in vitro* and *in vivo* (3–23). Specifically relevant to the methods described here are biopanning approaches used to characterize organ and cell homing peptides that can target the vasculature and parenchyma (3, 14–17, 24–27), peptides that can mediate transcytosis across epithelial cells *in vitro* (11), and antibodies that transmigrate phage across the blood-brain barrier (13). Our own laboratories' focus has been to identify and exploit ligands that internalize into target cells. To this end, we have re-engineered phage vectors for binding to mammalian cells (9) and monitored their entry into cells by immunostaining for internalization (28), transfection for drug delivery (7), PCR for DNA delivery (29) and transduction for gene expression (30).

Recently, we have been using these methods to evaluate whether it is possible to target the CNS and, specifically, whether the unique features of the choroid plexus (CP) at the interface between blood and cerebrospinal fluid (CSF) (31–36) would allow exploitation of molecular translocation into the CP, CSF, and brain parenchyma. To this end, we have used EGF-targeted phage (30) as a test substance to explore phage targeted to the CP nexus (Fig. 1). We reasoned that if EGF-phage could be targeted to the epithelium, then targeted-phage could be identified to assist in drug translocation directly to the CP. Alternatively, genes could be delivered to the CP to restore its function or to secrete therapeutic proteins into CSF (31–33, 37–42). If successful, the CP could be re-engineered to modulate its production of CSF and deliver biological agents to treat CNS disease (32, 36, 37, 43–49).

With cultured CP cells (Fig. 1), it is possible to demonstrate that particle internalization is dose dependant. In the example shown here, EGF-targeted phage were added in concentrations of 10^9–10^{11} to cells in culture and incubated as described below for 2 h before internalization analysis. Under these conditions, internalization of untargeted particles is nearly undetectable (Fig. 1a, c, e), but the EGF-targeted particle is readily detected inside the cultured CP cells (Fig. 1b, d, f). These data establish that CP cells in culture can be used to test targeting ligands and that the methods are applicable for biopanning libraries for CP targeting. In other studies, we used the methods described below to mine a phage display library to characterize novel targeting peptides that also target these cells (not shown).

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Using explants of CP cells, it is also possible to show specific epithelial cell targeting. The example shown in Fig. 1 illustrates how it is possible to demonstrate the specificity of internalization. When explants of CP (Fig. 1g) are incubated with phage, there is internalization if the particles are targeted with EGF (Fig. 1h). Very little targeting is observed when untargeted phage is added to the media. When cells are preincubated in the presence of EGF prior to the addition of EGF-targeted phage, the EGF-dependant internalization that is normally detected (Fig. 1j) is not observed (Fig. 1k). In other studies, we used the methods described below to mine a phage display library to characterize novel targeting peptides that can also target these cells (not shown).

To identify CP-CSF targeting peptides for these different applications of drug delivery, it is necessary to mine libraries of peptide sequences. With the establishment of target validation by EGF-targeted phage (Fig. 1), it is possible that libraries can be explored for various classes of CP-targeting peptides in several different screening assays (Fig. 2). They include (a) targeting the CP by *in vitro* screening for targeting peptides after injecting peptide libraries into mice, (b) targeting CP epithelium by biopanning CP explants ex vitro and even (c) evaluating particle translocation across the CP and into CSF. To explore the possibility of translocation across the CP and into CSF, PCR rather than biopanning can be used to detect particles in CSF after an intra-arterial injection of libraries into rat (Fig. 1l). However, because the concentration of recovered phage is very low and the sensitivity of the PCR is so high, the possibility of cross-contamination is a major concern. Still, these data point to possibly using combinatorial biology to identify transmigrating peptides *in vivo*.

Herein we describe how targeting can be screened for CP-targeting peptides using cultured CP cells, explants of intact CP, brain intracerebroventricular injections (i.c.v.), and CSF collection. Screening methodologies can be applied toward identifying CP-targeting peptides using these same approaches by analyzing particle internalization with phage display.

2. Materials

2.1. Peptide Libraries

1. Use either the PhD-7 display (#E8102L), the PhD-12 display (#E8111L), or the PhD-C7C display (#E8120S), (New England Biolabs, Ipswich, MA, USA) (see Note 1).

[1]Although an EGF-targeted phage made in our laboratory (8, 30) is described in this chapter, the availability of commercial peptide display libraries from companies such as New England Biolabs puts phage display into the hands of any laboratory with basic equipment for molecular biology. Vectors obtained from these suppliers can be used to construct personalized libraries with oligonucleotide sequences of different length, antibodies or even cDNA libraries. These methods are described extensively in several reviews (2). New England Biolabs offers three premade random peptide libraries, as well as the cloning vector M13KE for construction of custom libraries. The premade libraries consist of linear heptapeptide (Ph.D.-7) and dodecapeptide (Ph.D.-12) libraries, as well as a disulfide-constrained heptapeptide (Ph.D.-C7C) library. The randomized segment of the Ph.D.-C7C library is flanked by a pair of cysteine residues that are oxidized during phage assembly to a disulfide linkage, resulting in the displayed peptides being presented as loops. All of the libraries have complexities in excess of two billion independent clones. The randomized peptide sequences in all three libraries are expressed at the N-terminus of the minor coat protein pIII, resulting in a valency of five copies of the displayed peptide per virion. In both the Ph.D.-7 and the Ph.D.-12 libraries, the first residue of the peptide-pIII fusion is the first randomized position, while the first randomized position in the Ph.D.-C7C library is preceded by Ala-Cys. All of the libraries contain a short linker sequence (Gly-Gly-Gly-Ser) between the displayed peptide and the pIII protein sequence.

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2. Alternatively, phage can be engineered with known targeting ligands, e.g., epidermal growth factor (EGF), fibroblast growth factor (FGF), anti EGF receptor, ciliary neurotrophic factor (CNTF), and interleukin-2 IL2 (8, 20, 30, 50, 51).

2.2. Culture of Primary Choroid Plexus Epithelial Cells

1. Phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+}: 2.69 mM KCl, 1.47 mM KH_2PO_4, 8.1 mM Na_2HPO_4, pH 7.6.
2. 0.1 mg/mL Pronase (Sigma-Aldrich, St Louis, MO, USA).
3. 025% Trypsin (Invitrogen Inc, Carlsbad, CA, USA).
4. 5 μg/mL DNAseI (Roche Diagnostics, Indianapolis, IN, USA).
5. Ham's F-12 and DMEM (1:1) (Invitrogen Inc).
6. 10% Fetal bovine serum (Invitrogen Inc).
7. 2 mM Glutamine (Invitrogen Inc).
8. 50 μg/mL Gentamycin (Sigma-Aldrich).
9. 1 μg/mL Insulin (Sigma-Aldrich).
10. 5 μg/mL Transferrin (Sigma-Aldrich).
11. 5 ng/mL Sodium selenite (Sigma-Aldrich).
12. 10 ng/mL Epidermal growth factor (Sigma-Aldrich).
13. 2 μg/mL Hydrocortisone (Sigma-Aldrich).
14. 5 ng/mL Basic fibroblast growth factor (Invitrogen Inc).
15. Plastic tissue culture dishes (Falcon Laboratories, Colorado Springs, CO, USA).
16. Laminin (Boehringer-Ingelheim GmbH, Ingelheim, Germany).
17. Transwells (Corning, Lowell, MA, USA).

2.3. Dissection of CP

1. Donor Balb/c mice (4–6 week old).
2. 3–5% Isofluorane or suitable IACUC approved terminal anesthesia.
3. Surgical instruments for dissection: scalpel, scissors, and forceps.
4. Dissecting microscope.
5. Razor blade.

2.4. Explants of CP

1. Tissue culture 10 cm plastic dishes (Falcon).
2. Tissue culture 12, 48, and 96 well plastic dishes (Falcon).
3. Glass Pasteur pipette, modified for tissue harvesting (see Note 2).

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4. Ligand-targeted phagemid (10^{10}–10^{11} pfu/mL).
5. Dissected choroid plexus from Subheading 2.3 (1 CP/animal/well).
6. RPMI medium (Invitrogen Inc) containing 5% normal horse serum (NHS, Invitrogen Inc) and 10% fetal calf serum (FCS, Sigma-Aldrich).

2.5. Biopanning Peptide Libraries for CP Targeting

1. Peptide library (10^{10}–10^{11} particles, New England Biolabs) in culture medium incubation buffer.

2.6. Visualization of Phage Internalization

1. Rabbit anti-fd bacteriophage antibody 1:700 (Sigma-Aldrich). Control M13KE phage (New England Biolabs, N0316S) and ligand-targeted M13KE phage that are identified by biopanning.
2. Blocking solution: 5% NGS and 1% BSA in PBS.
3. Alexa 594-labeled goat anti-rabbit antibody (Invitrogen Inc): 1:1000 dilution in PBS/BSA and 1.5% NGS.
4. Mounting media containing DAPI (Vector Laboratories, Burlingame, USA).
5. 0.3% PBS-Tween 20.
6. Absolute methanol.
7. Normal goat serum (Vector Laboratories): 5% in PBS.
8. Bovine serum albumin (Jackson Immunoresearch Laboratories Inc, Westgrove, USA): 1% in PBS.
9. Fixative solution: 2% paraformaldehyde containing 2% glucose in PBS, pH 7.4.
10. Fluorescence Microscope.

2.7. Establishing Targeting Specificity

1. Prepare synthetic peptide (10–100 μg) that corresponds to the ligand sequence.
2. 50 mM HCl, pH 2.
3. 50 mM Glycine buffer pH 2.8 and 500 mM NaCl.
4. Anti-receptor antibody, 1:1000 (see Note 3).

2.8. Evaluating Intracerebroventricular Targeting In Vivo

1. Stereotaxic frame.

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[2]Glass Pasteur pipettes are modified for tissue harvesting by heating the end to form a loop-like structure that can be used to pull explanted tissue out of cell culture plates without damaging the tissue. Any similar device (e.g., small brush) is acceptable, but the use of forceps is discouraged because of tissue damage that leads to false positive staining. To avoid cross contamination, it is imperative that a different collection device be used for each sample. This is why the modified Pasteur pipette is the cheapest, most reliable alternative that does not cause damage to tissue.

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2. 50 uL Hamilton syringe.
3. Tissue-Tek OCT Solution (Sakura-America, Torrance, CA, USA).
4. Cryostat.

3. Methods

3.1. Peptide Libraries for Biopanning

1. Peptide libraries are handled as described by the manufacturer (see Note 1).
2. Aliquots containing 10^{11}–10^{12} pfu/mL are added to target CP cells in culture or CP explants obtained as described in Subheading 3.3.

3.2. In Vitro Biopanning Using Cultured Rat CP Cells

1. Dissect the CP under conventional light microscopy (see Note 4).
2. Rinse the tissue twice in Ca^{2+} and Mg^{2+} free PBS.
3. Digest 25 min with 0.1 mg/mL pronase at 37°C.
4. Recover predigested tissue by sedimentation.
5. Shake briefly in 0.025% trypsin containing 12.5 μg/mL DNAse-I.
6. Remove supernatant and keep on ice in 10% fetal bovine serum.
7. Repeat digestion five times.
8. Pellet cells by centrifugation.
9. Resuspend in full Ham's culture medium.
10. Incubate resuspended cells on plastic dishes for 2 h at 37°C.
11. Collect supernatant containing unattached cells.
12. Place medium for seeding on laminin-coated transwells.
13. Perform experiments after 7 days on confluent cell monolayers (see Note 5).

[3]Peptide specificity can be evaluated in many ways. The most effective is to preincubate the explants or cells in culture for 10–20 min at 4°C; however, ligand can also be added at the same time as the ligand-phage and incubated together with the cell targets at 37°C for 2 h. For the ex vivo studies, for example, the explants can also be incubated with the ligand-peptides for 40 min at 37°C under 5% CO_2 before beginning the incubation with the ligand-targeted phage. The approach is ligand dependant and should be changed according to the best approximation of the ligand–receptor interaction. If the targeting ligand's receptor is known (e.g., EGF targeting through EGF receptor), then antibodies to the receptor can be preincubated with cells. In either case, the signal detected by immunostaining will be decreased in co-incubated samples indicating that internalization is specific and receptor mediated.

[4]It is critical that the cerebellum be removed from the skull intact if the choroid plexus needs to be recovered from the fourth ventricle. To this end, the animal is killed with an overdose of anesthetic and the brain is carefully dissected out from the skull by making a midline incision in the skull, with care not to damage the cerebellum. A second incision is made in a coronal plane at the level of the orbit. With small forceps, the brain is separated from the skull, the optic and trigeminal nerves are cut and the brain placed in a petri dish containing cold PBS.

[5]The protocol for cell culture of rat CP derives from published methods (48) optimized for neonatal CP dissected from 3–6-day-old pups. However, cell lines (52) are also compatible with the experimental approach of ligand targeting. Once established, the upper chamber of cultured epithelial cells represents the fluid in contact with the apical side of epithelial cells (the CSF side), while the lower chamber represents the basolateral side (the blood side).

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3.3. Ex Vivo Biopanning Using Explants of Mouse or Rat CP

1. Terminal anesthesia for 4–6 week mice (or rats) is performed using methods approved by the Institutional Animal Care and Use Committee of the University of California (IACUC) (see Note 6).
2. Dissect brains from the skull (see Note 4).
3. Harvest CP from the fourth ventricle (see Note 7) and lateral ventricles (see Note 8).
4. Place fragments of CP in 1 mL of RPMI media containing 5% NHS and 10% FCS.

3.4. Collection of CSF

1. Immobilize a deeply anaesthetized rat in a stereotaxic frame.
2. Raise the head above the body so that the head is elevated but parallel to the table top.
3. Make a central incision overlying the top of the skull to the base of the neck.
4. Expose the posterior aspect of the skull using blunt ended scissors.
5. Retract muscle and fascia.
6. Observe landmarks of bone (white and hard), separation of bony area (white line), and the presence of a 2–5 mm circular yellow/white membrane at their intersection.
7. Perforate membrane with a 25–100 uL Hamilton syringe.
8. Withdraw CSF slowly and place in a cryovial and freeze.

3.5. CP Dissection After In Vivo Biopanning

1. Perform terminal anesthesia on mice using methods approved by the IACUC (see Note 6).
2. Dissect brain from the skull (see Note 4).
3. Harvest choroid plexuses from the fourth ventricle (see Note 7) and lateral ventricles (see Note 8).

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[6]Isofluorane or halothane overdose appears suitable for CP dissection and yields choroidal tissues compatible for analysis. Nontraumatic sacrifice prevents hemorrhage in the brain upon decapitation and dissection although the effects of anesthesia on CP function deserve consideration. Direct decapitation is discouraged because of the hemorrhage in the brain and blood in the microvasculature of the CP.

[7]The cerebellum is carefully separated from the rest of the brain. The anterior portion of the cerebellum is placed facing down on a Petri dish (Fig. 3a). Under a stereomicroscope and with the aid of two pairs of tweezers, the cerebellum is carefully lifted and separated from the brain stem to visualize the fourth ventricle. The CP, resembling a "Y" attached to the roof of the cerebellum, can then be easily dislodged with tweezers. The CP is placed in RPMI media containing 10% FCS and 5% NHS.

[8]The lateral ventricle CP is dissected after removal of the fourth ventricle CP. The brain is immersed in PBS, and two parallel sagittal incisions are made with a scalpel 10 mm from the midline along the length of the brain and to a depth of 4 mm in such a way that it cuts through the corpus callosum (Fig. 3b). The cortex is then pulled away to the side exposing the lateral ventricles and choroid plexuses. With a pair of tweezers, each end of the CP is gently pulled away. The CP is then placed in RPMI media containing 10% FCS and 5% NHS.

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3.6. Biopanning, Recovery, and Amplification of Targeted Phage

1. Dilute phage to desired concentration (10^9–10^{12} particles/mL) in culture media.
2. Add particles to target cells or explants (see Note 9).
3. Incubate cells (or explants) for 2 h at 37°C in a CO_2 tissue culture incubator.
4. Wash cells (or explants) 3–5 times with PBS containing Ca^{+2} and Mg^{+2}.
5. Incubate cells (or explants) with 50 mM glycine buffer pH 2.8 containing 0.5 M NaCl for 5 min at room temperature and process for immunostaining.

3.7. Detection of Internalized Phage (see Note 10)

1. Transfer CP fragments to individual wells of a 96 well plate containing 100 uL RPMI with 10% FCS and 5% NHS.
2. Add phage (purified or library) in 10 μL to each well.
3. Incubate for 2 h in 37°C incubator under 5% CO_2.
4. Add 100–200 μL PBS with Ca^{+2} and Mg^{+2} to each well (see Note 11).
5. Using a clean glass scoop (see Note 2), transfer CP to the PBS wells of a previously prepared 48 well plate containing 1 mL of PBS (see Note 12).
6. Move one piece at a time and when all tissues are moved, transfer them to the next wash step (<1 min/step).
7. Repeat cycle through PBS two changes, PBS/Tween three changes, PBS three changes, and fix tissue in the fixative solution at room temperature for 20 min.
8. Wash once with PBS and transfer to methanol for permeabilization for 10 min.
9. Wash twice with PBS.
10. Transfer to a blocking solution of 5% NGS/1% BSA in PBS and incubate for 20 min at room temperature.
11. Transfer to rabbit anti-M13 (fd) for 1 h at room temperature.
12. Wash twice with PBS and transfer to Alexa 594 labeled goat anti-rabbit antibody for 45 min at room temperature.

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[9]In order to evaluate whether the peptides identified by biopanning epithelial cells in culture or explants are active (and specific) *in vivo*, it is important that they be tested by injecting peptide-targeted phage i.c.v. and subsequently examining whether they can enter the CP epithelium, ependyma, and even brain parenchyma. Immunohistochemical staining for M13 coat protein is used to detect the distribution of particles throughout the brain.

[10]There are several ways to monitor internalization of phage. The technique described here is immunofluorescence. Because the display peptide ligand enables internalization of the particles into endosomes of the target cells, the major coat protein (pVIII) can be visualized in permeabilized cells for up to 24 h after entry into cell. Later, the coat protein is found to be degraded so that immunostaining is not possible. In contrast, the internalized phage DNA appears to have lived much longer than the coat protein and it can be used for recovery and identification of targeting agents. We have found phage DNA in cells 3 months after internalization *in vitro*.

[11]The PBS is added to wells to make it easier to collect the dissected tissue from the well and transfer it to the next treatment.

[12]It is recommended to pre-prepare a template for washes depending on the size of the experiment (Fig. 4). In the example given here, a 48 well plate is prepared with the washes and incubations that are necessary for two treatments. Identical treatments can be combined for each of the steps without compromising data quality. It is most critical not to damage tissue during transfer.

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13. Wash twice with PBS, and PBS-DAPI prepared by adding one drop of DAPI to mounting medium in 100 uL PBS.
14. Mount tissue on slide with a fine brush and place a coverslip over the tissue.
15. Evaluate immunostaining by fluorescence microscopy.

3.8. Evaluation of Targeting Specificity

1. Prepare synthetic peptide (10–100 μg) that corresponds to the sequence identified by phage display in 50 mM HCl, pH 2.
2. Incubate with cells (or target).
3. Add phage (10^{10}) to target.
4. Incubate as given in Subheading 3.6, step 3.
5. Process for internalization.

3.9. Evaluating Intracerebroventricular Targeting In Vivo

1. Immobilize the head of the deeply anesthetized rat (200–250 gm) in stereotaxic apparatus.
2. Make a midline incision in the skin overlying the skull and identify the bregma on the skull surface.
3. Using a microdrill, perform a craniotomy at the following coordinates: 1 mm posterior to the bregma and 1.5 mm lateral to the midline.
4. Insert the needle attached to a glass Hamilton syringe through the craniotomy into the brain to a depth of 4 mm and inject 20 μL of the phage solution into the lateral ventricle (see Note 9).
5. Leave the needle in place for 1–2 min to avoid reflux of the injection fluid.
6. At specific times after injection (24–72 h), kill deeply anesthetized the animals using techniques approved by local Institutional Animal Care Committee such as overdose or CO_2 inhalation.
7. Perfuse the animals with 4% paraformaldehyde in PBS, pH 7.4 via a cannula in the left ventricle.
8. Dissect the brain out and postfix overnight at 4°C in 4% paraformaldehyde in PBS.
9. Place brain in 30% sucrose overnight.
10. Embed in OCT compound and store at −80°C.
11. Mount 12 μm-thick cryostat sections onto positively charged slides.
12. Perform immunostaining.

3.10. Evaluating Targeting In Vivo

1. Inject 100 μL of phage into a tail vein or carotid artery (see Note 13).

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2. Harvest CP as described above in Subheading 2.3.

3. Perform immunostaining.

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[13]Because the biopanning protocols described here identify epithelial targeting *in vitro* and ex vivo, they will be suitable for systemic drug delivery if they can translocate across the permeable choroidal endothelium (likely) and enter epithelial cells from the basolateral (blood) side of the barrier. Particles (10^{12} in 100 μL PBS) are injected intra-arterially and CSF collected for PCR analyses of phage DNA.

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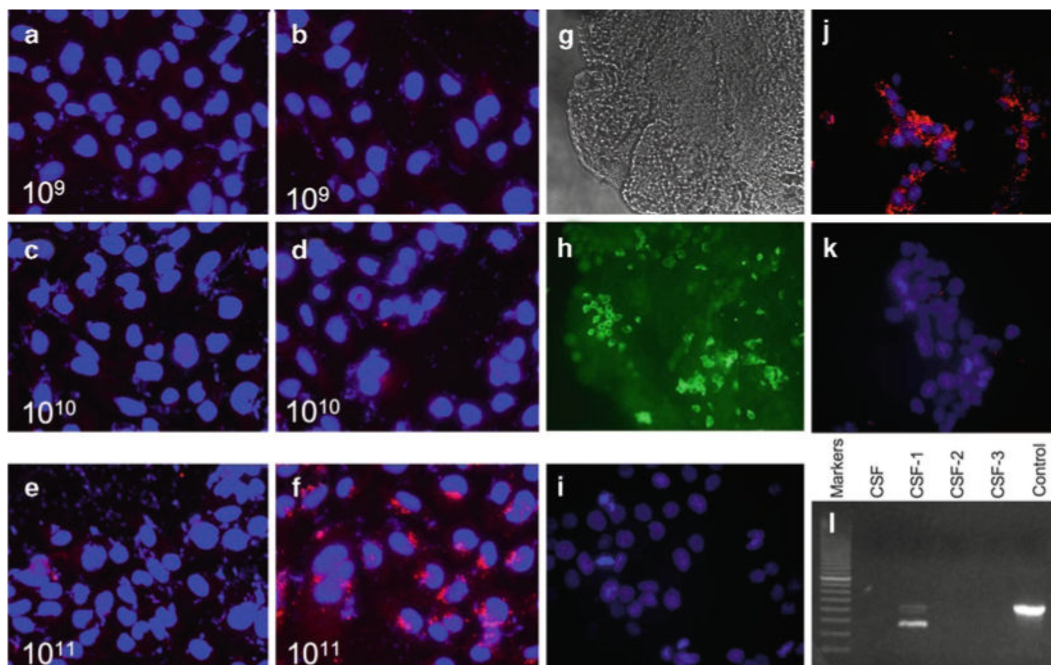

Fig. 1.

Targeting cultured CP epithelial cells and explants with EGF phage is shown. Using the methods described here, control (untargeted phage) and EGF-targeted phage were incubated with either cultured CP epithelial cells (**a**–**f**), explants of mouse CP (**k**), or injected i.v. (**i**) to demonstrate the feasibility of CP targeting with phage. Controls (**a**, **c**, **e** and **i**) used untargeted phage, whereas EGF-targeted phage was evaluated in **b**, **d**, **f**, **h** and **j**. When the particles are added to cells after adding exogenous EGF (1 μg/mL), then specificity can be demonstrated by eliminating the internalization (**k**). (**l**) In another approach, PCR can be used to assess recoveries from CSF in control untreated animals (CSF) and in three treatment groups (CSF-1, CSF-2, and CSF-3) as long as there is a positive control.

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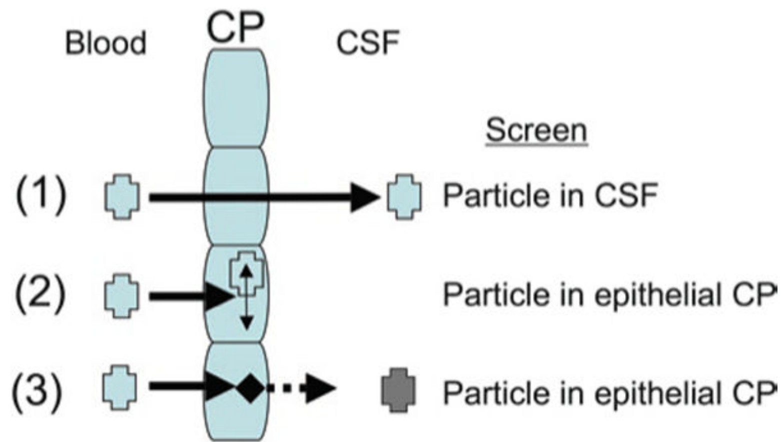

Fig. 2.
Strategies for drug targeting to the CP are shown. There are three ways to target drugs to the brain via the CP. Firstly, drugs could be translocated directly into CSF from the apical (ventricular) side of the epithelium to find their targets via CSF bulk flow. In the second mechanism, the CP itself is the drug target, and the epithelium could be the therapeutic target of the drug and modulate its natural functions. The third mechanism targets the CP, for example with a gene, with the goal of exploiting the CPs natural ability to produce CSF and secretes biotherapeutic factors. Phage display could be used to identify each of the three categories of CP targeting agents depending on the different biopanning screens deployed. In the first, the CP is targeted "transchoroidally" from blood to CSF, in the second, it is targeted to the basolateral (blood-facing) epithelium, and in the third to the apical (CSF-facing) epithelium.

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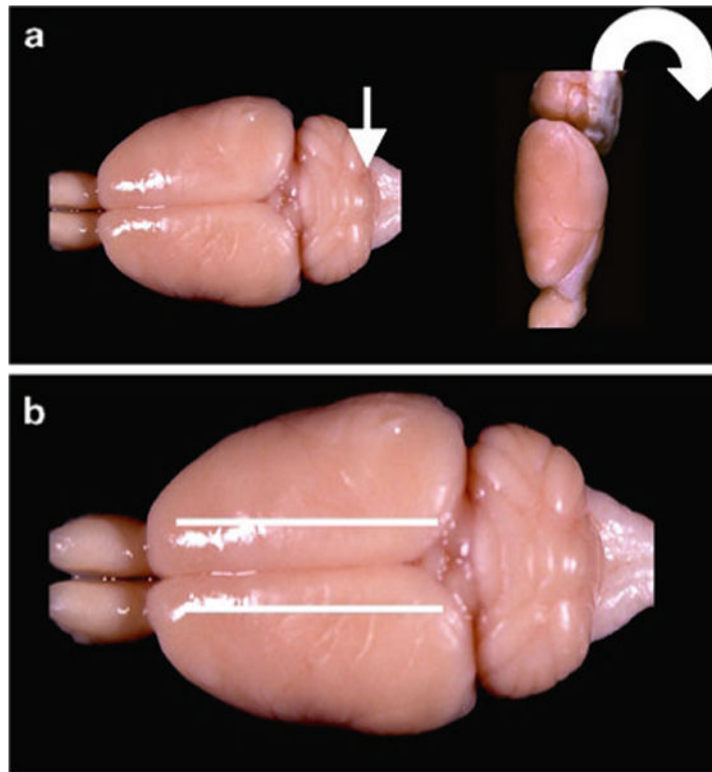

Fig. 3.

(**a**, **b**) Dissection of brain for choroid plexus sampling: Three cuts with a flat edged razor blade enable dissection of the CP from the brain. While it is possible to accomplish this on the bench top, a dissecting microscope is highly recommended.

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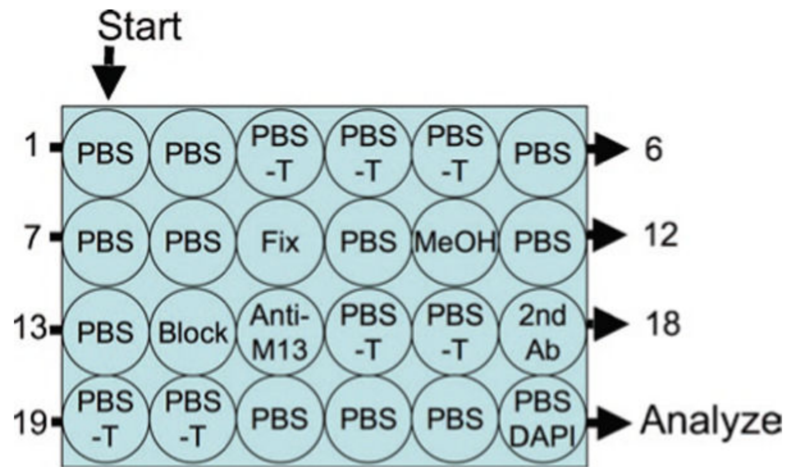

Fig. 4.

Template for immunostaining. A 24 well plate is prepared prior to immunostaining using a template like the one shown here. Tissues are carefully transferred from one well to the next rather than washing in a single well and risking losing tissue.