Hitting the Target with Bifunctional Phage

Peptide libraries are rich sources of cell-targeting peptides. In this issue of *Chemistry & Biology*, Pasqualini and coworkers have developed a chimeric cell-targeting phage system that can easily be modified to delivery of a variety of reagents without need for chemical conjugation [1].

Despite the fact that Paul Ehrlich introduced the concept of the "magic bullet drug" almost 100 years ago, cell-specific targeting of therapeutic molecules remains a major challenge in biomedicine. Most efforts toward this goal have focused on exploiting monoclonal antibodies that recognize well-characterized cell surface antigens as cell-homing reagents. While the use of monoclonal antibodies as delivery reagents has the advantage of high affinity and specificity, the approach suffers from certain limitations, such as nonspecific uptake by the reticuloendothelial system, high production costs, and difficulty in modifying these macromolecules to deliver active therapeutics.

To overcome the limitations of antibodies, chemists and biologists have turned to peptides as targeting agents [2, 3]. Phage display libraries have proven to be a rich source of cell-targeting peptides. Phage panning on cell surface receptors [4, 5], cells in culture [6–12], and in whole animals [13–16] has yielded peptides that bind cells with good affinities. More importantly, many of the isolated peptides display specificity for the target cell type, indicating that they may be useful reagents for clinical applications.

Biopanning on intact cells in culture or in whole animals has the important advantage that it requires no prior knowledge of any cell-specific receptor. Thus, peptides can be isolated that target poorly characterized cell types, where little is known about their cell surface protein profile. Pasqualini and coworkers have pioneered the use of in vivo panning to isolate organ homing phage from peptide libraries [13]. In summary, with this method phage libraries are injected into the tail vein of mice. After a short incubation time, the mice are sacrificed, and the target organs of interest are removed. The organ-associated phage are retrieved and amplified from the homogenized tissue, and the panning is repeated in another mouse using the isolated phage pool. After 3–5 sequential rounds of panning, among the isolated organ-specific peptides, several peptide motifs are typically identified for a given organ. Using this protocol, phage have been isolated that target the vasculature of many different tissue types as well as different tumors [13–16]. This group has recently reported progress toward isolating peptides that home to vasculature beds in humans as well [17].

Once a phage clone that targets the desired cell type has been isolated, the next challenge faced by researchers is to utilize it for delivering active biomolecules, such as therapeutics, imaging agents, genes, and proteins. While bacteriophage can be employed to deliver DNA to mammalian cells, problems are encountered when using the phage particles for delivery of other reagents [18]. Like antibodies, bacteriophage are large and difficult to modify in a regiospecific fashion. Often, chemical coupling of small molecules or proteins to the surface of a phage results in loss of binding to the cellular target, the formation of phage aggregates, and/or a decrease in the ability of the phage to infect *E. coli*. For these reasons, efforts have been placed on translating the peptide responsible for cell targeting out of the context of the phage particle itself, while retaining the peptide's affinity and cell-targeting abilities. The resulting synthetic peptide constructs have been used to deliver small molecules into cells, and adenovirus particles have been modified with synthetic cell-specific peptides to redirect the native tropism. However, there are drawbacks to this approach, including the requirement for a great deal of experimental optimization. Thus, this is not a convenient method for rapid validation of phage clones isolated from phage display selections.

In this issue of *Chemistry & Biology*, Arap and Pasqualini report the construction of a chimeric phage that can easily be modified for the delivery of a wide variety of reagents [1]. Taking advantage of established methods to display foreign peptides on the surface of filamentous bacteriophage, the group genetically engineered a M13 bacteriophage derivative to display a cell-targeting peptide on the pIII minor coat protein, as well as a biotin-like peptide on the pVIII major coat protein (Figure 1). The cell targeting peptide is a well-characterized double-cyclic peptide comprised of the sequence CDRGDCFC and is referred to as RGD-4C. RGD-4C was originally selected from a phage panning experiment using the $\alpha_v\beta_3$ integrin as bait [4] and was again selected in an in vivo panning experiment to isolate peptides that target the vasculature of breast tumors [19]. Subsequently, RGD-4C was shown to bind to α_v integrins and consequently selectively home toward several different types of tumors displaying this integrin subunit [20]. The biotin-mimetic peptide used by the Pasqualini group contains the streptavidin binding motif HPQ and is displayed on approximately 4%–8% of the pVIII proteins on the phage surface. The authors demonstrate that the hybrid phage retains its affinity for both its cellular target, α_v, and streptavidin.

Addition of the biotin-like tag to the phage scaffold allows for the phage to be characterized in several different applications without further chemical modification. In this report, the authors demonstrate that the chimeric phage can be attached to a streptavidin-coated solid support (plates or magnetic beads) and used to capture α_v-expressing cells. Phage that express only the RDG-4C peptide or the biotin-like peptide alone could not successfully capture and retain α_v-expressing cells, indicating that the phage binds to the solid support through the biotin-mimetic peptide, and the RGD-4C peptide is responsible for cell binding. Furthermore, the authors demonstrate that the biotin peptide can be used in a similar fashion to biotin in cell-staining experiments. To visualize phage binding in their study, chimeric phage

Figure 1. Graphic Representation of a Bifunctional Cell-Targeting Bacteriophage

were incubated with cells followed by subsequent incubation with streptavidin-coated fluorescent microspheres. The fluorescent streptavidin beads bound the biotin peptide-labeled phage, illuminating cells with bound phage particles. Cell-targeting hybrid phage can also be utilized to deliver fluorescent nanoparticles in a cell-specific fashion. The authors demonstrated this by conjugating chimeric phage to streptavidin-coated quantum dots via the biotin peptide. The nanoparticle was successfully delivered to the cytoplasm of cells expressing α_v integrins; thus, not only is the phage internalized, but it can deliver cargo as well. Lastly, the chimeric phage was utilized in a surface plasmon resonance assay to determine cell binding. Streptavidin-coated biosensor chips were coated with the hybrid phage, and target cells were passed over the sensor to detect cell binding over time. Consistent with earlier experiments, only background binding was observed when no phage is added to the sensor chip, and the rate of cell mass buildup is significantly greater than when the control phage is used. In summary, addition of the biotin peptide to the surface of the phage allows for many different in vitro assays to be performed to assess cell binding. While many of these assays could be performed in other ways (for example, cell staining using anti-phage antibodies), the biotin-peptide tag allows for systematic characterization of the phage clone without extensive development of new reagents and/or assays.

In vivo applications are expected to be much more demanding on the chimeric phage. First, stringent cell specificity of the targeting phage is critical, as phage will encounter many different cell types in this environment, some in much larger numbers than the desired target. Second, the noncovalent streptavidin-phage complex must be stable under physiological conditions. To test the utility of the chimera phage for in vivo delivery, the authors demonstrated the ability to the chimeric phage to deliver a quantum dot to a tumor in an animal model. While the tumor specificity of the phage is not dramatic (tumor/brain ratio of 25 for the targeted phage and 6.5 for the control phage), and there is substantial nonspecific uptake of the phage in the liver, these experiments clearly demonstrate the utility of the bifunctional phage as a rapid way to validate phage clone leads in animal models.

This proof-of-concept paper elegantly illustrates how fusing a peptide tag to the phage scaffold can allow for many different assays to be performed without the need for chemical conjugation or the creation of new reagents. While other chimeric phage have been created [21, 22], none have been so thoroughly characterized and have the broad applications of this construct. The power of this type of phage construct is its utility for rapidly validating of phage clones isolated from library selections. Creating chimeric phage library in which members of a peptide library are expressed on pIII and the biotin peptide on pVIII will allow for rapid selection and characterization of phage clones. While there will certainly continue to be efforts placed on translating the translating peptides out of the context of phage to be used as small molecule delivery reagents, this dual-function phage will allow the peptide leads to be prescreened as delivery reagents before further development as potential therapeutics.

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Selected Reading

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