NANOMEDICINES

Laura Sánchez-García*
Universitat Autònoma de Barcelona

INTRODUCTION

Nowadays, the prevalence of cancer is a worldwide public health burden. The current treatments suffer from a lack of specificity and selectivity. For that reason, it is necessary to provide alternatives to target cancer treatment to malignant cells exclusively and make it become less aggressive.

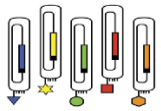

Figure 1 Phage display library. This illustration represents the linkage between genotype and phenotype (Adapted from 1).

The best alternative is **phage display,** a method for presenting polypeptides on the surface of bacteriophages. This technology is based on a direct linkage between phage phenotype and its encapsulated genotype, which leads to presentation of molecule libraries fused with the coat proteins on the phage surface. The phage display technique allows the creation of libraries (Fig. 1) which contain up to 10^{10} different variants.

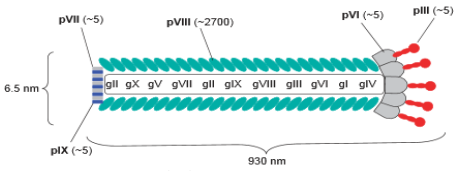

Figure 2 Structure of a filamentous bacteriophage particle (the copy number of each protein is shown in brackets) (2).

The aim of this review is to provide the necessary knowledge to understand the basis and potential of the phage display technique. Furthermore, it focuses on the development of a targeted drug therapy against cancer cells using the specific peptides obtained by phage display.

MATERIAL AND METHODS

Library construction

- ✓ **Choice of phage:** filamentous (Fig. 2), T4, T7 or lambda
- ✓ **Type of coat protein:** there are three structural proteins on M13 that have been used as vehicles for peptide libraries. The most common by far is pIII, followed by pVIII (Fig. 2)
- ✓ **Type of ligand displayed:** peptide libraries or antibody libraries
- ✓ **Full or hybrid display:** it determines whether all the copies of the coat protein will display the ligand (full display) or only a few of them (hybrid display).
- ✓ **Insert location:** the mutant gene can be encoded in the phage genome or in a phagemid (Fig. 3).

Taking into account all those parameters, it will be possible to develop different types of display (shown in Table 1).

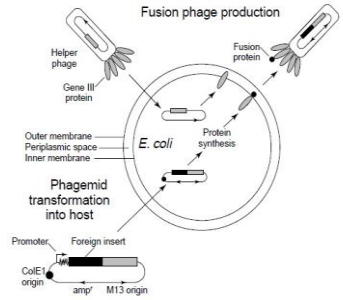

Figure 3 Use of a phagemid vector and a helper phage to produce a hybrid display (the black square represents the gene that encodes the ligand and the black circle represents the ligand displayed) (Adapted from 3).

Screening and selection

The screening method (Fig. 4) enriches the phage clones that bind to the target of interest by a process called biopanning (4, 5), which involves the following steps:

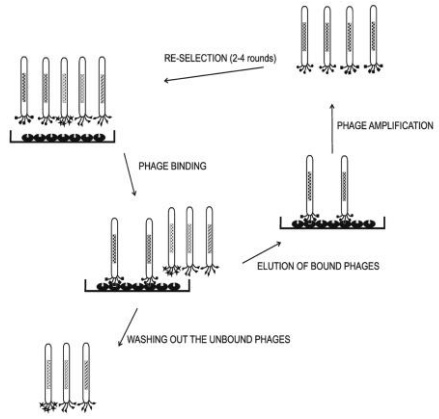

Figure 4. Representation of the biopanning process designed to enrich the phage that binds specifically to the target. All the steps must be repeated three to six times, until sufficient enrichment has occurred (Adapted from 6)

1. Target immobilization: for example in a solid support (96-well polystyrene microtiter plates).
2. Phage binding: the library is added to the target coated well.
3. Washing: unbound bacteriophages are removed. The first round of biopanning requires higher yield of the fittest phage clones over the background and hence the washes are less stringent.
4. Phage elution: adding for example a solution containing either free target or a competing ligand.

Finally, the eluted phage is amplified in bacteria (by infecting *Escherichia coli*). The phage clones obtained are analysed by DNA sequencing to identify the target binding peptides.

Table 1 Classification of most of the common phage display vectors (7)

DISPLAY TYPE	COAT PROTEIN DISPLAYED	FULL OR HYBRID DISPLAY	FUSION POSITION
Type 3	pIII	Full	Phage genome
Type 8	pVIII	Full	Phage genome
Type 33	pIII	Hybrid	Phage genome
Type 88	pVIII	Hybrid	Phage genome
Type 3+3	pIII	Hybrid	Phagemid
Type 8+8	pVIII	Hybrid	Phagemid

RESULTS

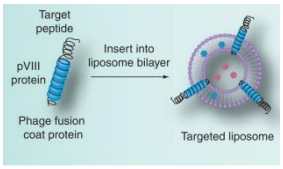

Figure 5 Production of a targeted liposome by insertion of a phage fusion coat protein into its membrane. The pink circles placed in the hydrophilic pocket represent the drug administered, in this case doxorubicin (8).

Peptides isolated from phage-displayed libraries can be used as targeting molecules for many applications: radiolabeled peptides, peptides conjugated with chemotherapeutic agents and peptides on nanoparticles or liposomes carrying chemotherapeutic agents (5).

For instance, doxorubicin-loaded liposomes (Doxil) have been modified with a phage fusion coat protein specific towards MCF-7 breast cancer cells (Fig. 5). MCF-7-phage-Doxil demonstrated a significantly enhanced association with target cells and an increased cytotoxicity. In conclusion, the incorporation of such phage protein into doxorubicin-loaded liposomes resulted in a remarkable increase in the killing efficiency of targeted tumour cells.

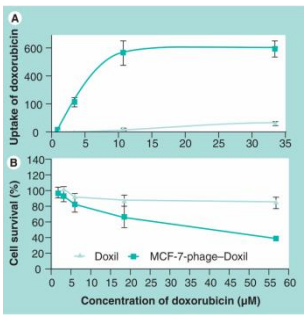

Figure 6 Correlation between doxorubicin uptake and cytotoxicity of Doxil ® and MCF-7-phage-Doxil. **(A)** Uptake of doxorubicin by MCF-7 cells. **(B)** Cytotoxicity of Doxil and MCF-7-phage–Doxil towards MCF-7 cells (8).

CONCLUSIONS

•Phage display is a valuable tool in biomedical applications which offers rapid, efficient and relatively inexpensive methods. The outstanding advantage of this technique is the ability to generate an enormously diverse exogenous peptides or proteins displayed on the surface of the phage using standard rapid molecular methods.
•Peptide-mediated targeting liposomes offer several advantages over the use of free drugs in treatment of breast cancer. Furthermore, the modification of existing liposomes with phage protein provides liposomes with the ability to bind target cells stronger compared to the non-modified liposomes.
•To sum up, phage display is a powerful technique that can be very useful in the near future, although it has to be studied in depth in order to improve some weak points.

References

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