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Lambda Phage Nanoparticles for Targetomics

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Abstract: The emerging aim of drug delivery requires the bioactive or therapeutic molecule to be protected from degradation and reach its target cell and intracellular location. Target specificity of nanoparticles is a prerequisite to attain the concentration of therapeutic agent required for therapeutic efficacy in the target tissue while minimising adverse effects on other parts of the body. Therefore, there is an urgent need for improvement of more effective drug delivery systems to direct the anticancer drugs to cancer cells, specifically. In the paper, we have described advantages of Lambda bacteriophage over other drug delivery vectors and proposed it as promising drug delivery vehicle.

Key words: Lambda bacteriophage, drug delivery, targetomics, cancer therapy, phage display

INTRODUCTION

The targeted therapy, targetomics, is attractive concept because it demonstrates some of the advantages of topical application of drugs: high local concentration and low systemic exposure. Cancer stands out as a disease most likely to benefit from targetomics.

Studies have demonstrated that some of antigenic markers on surface of tumor cell can be used as molecular targets. (Sergeeva *et al*., 2006; Ghaemi *et al*., 2007; Saraf *et al*., 2011a). Efficient drug delivery systems are required for targeting to specific tumor regions. Therefore, the application of targeted drug delivery systems is set to developed (Barton and Waxman, 1990; Krishnan *et al*., 2010; Balamuralidhara *et al*., 2011).

Nanoparticles as drug delivery systems, with cell-specific targeting, can overcome some limitations of conventional cancer treatment techniques (Ehdaie, 2007; Jain, 2005; Harisa *et al*., 2011).

Pharmaceutical nano-based particles have many advantages over conventional drug delivery systems but it has been realized that preparation of these nanocarrier system involves use of harsh toxic solvents in the production process (Mainardes *et al*., 2005; De Jong and Borm, 2008; Saraf *et al*., 2011b). Furthermore, the particles may initiate immunological side effects and allergic reactions (Kim, 2003; Varde and Pack, 2004; Elsayed *et al*., 2006).

Phage display and targeting: Recently, different potential applications have been identified for bacteriophages in pharmaceutical biotechnology (Jepson *et al*., 2004). Furthermore, the nanoparticles applications have been shown as gene delivery carrier and phage display technology (Petty *et al*., 2007; Heng *et al*., 2007).

The ability to display tumor recognition molecules on the phage coat enables targeting of specific tumor, a prerequisite for effective therapy (Rajotte *et al*., 1998; Heng *et al*., 2007).

In phage display, a heterologous peptide or protein is displayed on the surface of the phage through transcriptional fusion with a coat-protein gene, producing novel nanoparticles that have a variety of potential uses (Benhar, 2001; Zamit *et al*., 2010).

The physical linkage of the phenotype of a polypeptide to its corresponding genotype present within

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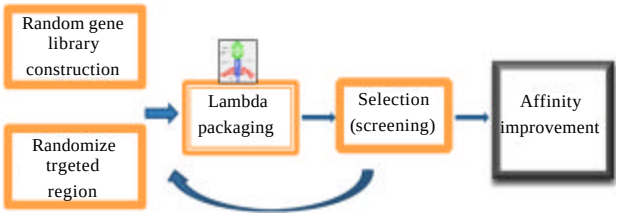

Fig. 1: Overall strategy for phage display to obtain high affinity ligands for binding to target molecule

the phage particle allows further manipulation of the ligand encoding genes to achieve the desired targeting entity. For drug targeting applications, where high affinity ligands are needed which specifically recognize surface tumor-associated antigens, this technology may be of great importance (Willats, 2002; Saxena and Moorthy, 2007).

The creation of ligand-displaying libraries in combination with powerful selection methods has opened up a wide range of possibilities not only for the search and generation of new ligands amenable for drug targeting but also in the field of drug discovery and drug design (Paschke, 2006; Landon *et al*., 2004; Al-Zubairi and Eid, 2010).

The most widely used phage display methods are based on the use of M13 and related filamentous phages of Escherichia coli but others, including the *E. coli* phages lambda and T7, have also been used (Clark and March, 2006).

Many features give phage display technology clear advantages over other approaches for the generation of platforms for targetomics. These include diversity in protein type and sequence space of combinatorial phage libraries, the possibilities of genetic manipulation to generate more effective ligands and specific effector functions and the adaptability of the system for the production of therapeutic ligands derived from the libraries (Ehrlich *et al*., 2000; Castagnoli *et al*., 2001; Ghaemi *et al*., 2012).

Lambda phage as drug delivery system: Since we strongly believe in ability of phage nanocarrier for targeted therapy, we have hypothesized novel engineered tumor-targeted lambda bacteriophages with controlled specificity suitable for targeted delivery of pharmaceuticals.

Despite of nanotechnology promise for drug targeting, preparation of targeting ligands, such as antibodies and their conjugation to lipids to make useful quantities of the targets of carriers has proven troublesome, differing idiosyncratically from one targeted particle to another (Gao *et al*., 1999). Accordingly, there is a need for an easily assembled targeted particle that has efficient assembly and conjugation, little bioreactivity and specificity and sensitivity in binding target sites.

The filamentous bacteriophage M13, with which was introduced the concept of display system is to date the most widely used system for display of peptides and proteins. Fusions to the minor coat protein, gIIIp and major coat protein, gVIIIp have been used for display of a range of molecules of different sizes and structure (Benhar, 2001). However, M13 morphogenesis occurs in the periplasm, therefore, it is essential that the molecules to be displayed be secretion competent. Because of this, it has been suggested that filamentous phage display libraries often do not display a number of possible fusions and for making full-length cDNA libraries from polyA mRNA (Chaudhary *et al*., 2008).

One system which obviates these problems associated with M13 display, is lambda display system (Fig. 1; Phage display strategy).

Lambda phages are assembled in the cytoplasm and lyse host for release. As a result, displayed peptides and proteins do not need to be capable of secretion through the host cell membrane (Ehrlich *et al*., 2000). Therefore, lambda phage vectors allows for the display of peptides and proteins that could not be displayed using filamentous phage vectors. These vectors allow for both high and low copy display, making it possible for low and high affinity binders (Vaccaro *et al*., 2006; Gupta *et al*., 2003). Flexible capabilities of bacteriophages lambda is important in its development as a delivery vector capable of targeting mammalian receptors.

Phage as a gene delivery system was developed following studies that identified "internalizing phages" from libraries of phage-displayed antibodies or peptides (Petty *et al*., 2007; Gao *et al*., 2003).

Our group has exploited the potential of lambda phage as a novel gene transfer vector for applications in vaccination and gene delivery, because of its favorable

safety profile (compared with mammalian virus vectors) and the ease and scalability of vector production (Ghaemi *et al*., 2010; Ghaemi *et al*., 2011).

Recently the potential of filamentous phages were exploited for targeted delivery by applying them as anti bacterial nanomedicines (Bar *et al*., 2008). Another study demonstrated a proof of principle of targeted, drug-carrying filamentous bacteriophage as anti-cancer agents (Yacoby *et al*., 2007).

According to above research and due to inexpensive production, suitability for large scale production, their physical stability, inability to cause human disease and compatibility with simple storage and finally lambda phage display advantages over other phage vectors, a hypothesis is generated for using lambda phage vectors as drug delivery vehicle.

Phage vectors that target to a to specific mammalian cell surface receptors such as inclusion of the integrin targeting peptide can efficiently internalized into mammalian cell. A large payload of drug are conjugated via a organic chemical linkage or genetic engineered cleavable linkers, proposing that lambda vectors might allow for efficient intracellular drug release into cancerous cells. Linkers have devised to maximize the drug payload per targeting molecule that binds a target site (Doronina *et al*., 2006; Liu, 2006).

Targeting of Lambda-bacteriophage nanocarriers to specific receptors on cancer cell membranes result in endocytosis, intracellular degradation and finally drug release, leading to growth inhibition of the target cells.

DISCUSSION AND CONCLUSION

Previous studies have described that a targeted drug delivery requires advantages of topical application of drugs such as high local concentration and low systemic exposure. An efficient drug delivery candidate should fulfill principles of target binding selectivity, large drug-carrying capacity and timely drug release at the target. Among different delivery systems, we have offered lambda bacteriophages as a versatile carrier to meet these criteria. Bacteriophage-based vectors have many of the desirable properties of both animal viral and non-viral systems without any significant drawbacks. Much like non-viral vectors, phage particles are simple genetic packages lacking intrinsic tropism for animal cell. However, phage can be produced at high titers in bacterial culture potentially making the production simpler and more economical than either nonviral or viral systems. Phage particles are also extremely stable under a variety of harsh conditions.

In addition, phages has been experimentally administrated to animals and used in safe manner for applications that include the treatment of bacterial infections and more recently immunization in humans.

Phages can also be designed to display specific ligands on their surface. The ligand libraries can be screened with a specific receptor biopanning to isolate the best ligand. The ability makes phage promising drug delivery system over other delivery systems.

To the best of our knowledge, few of the current drug delivery systems can distinguish tumor cells from normal cells due to the lack of the specific targeting, therefore, we hypothesized that lambda-bacteriophage nanoparticles can be modified for displaying specific ligands that will target these vectors to desired receptors of carcinoma cells, effectively. Targeted drug-carrying lambda-bacteriophage nanocarriers can internalize into the cells and release the drugs within them. The advantages of Lambda bacteriophage over other delivery systems have proposed lambda nanoparticles as a potential drug delivery system.

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