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Research review paper

Modified phages: Novel antimicrobial agents to combat infectious diseases

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ABSTRACT

Researchers increasingly believe that microbial, molecular and synthetic biology techniques along with genetic engineering will facilitate the treatment of persistent infectious diseases. However, such therapy has been plagued by the emergence of antibiotic-resistant bacteria, resulting in significant obstacles to treatment. Phage therapy is one promising alternative to antibiotics, especially now that recent modifications to ubiquitous phages have made them more controllable. Additionally, convincing in vitro and in vivo studies of genetically modified lytic phages and engineered non-lytic phages have confirmed the advantages of novel, specific bactericidal agents over antibiotics in some cases. There is still a need for a better understanding of phage therapy, however, before it can be adopted widely.

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Contents

1. Introduction

One of the main challenges in the post-antibiotic era of medicine is the prevalence of pathogenic, antibiotic-resistant bacteria (Carson and Riley, 2003; Nikaido, 2009). Traditional tactics as well as newer genomic mining techniques have not yet yielded novel classes of effective antibacterial compounds, so modern medicine has had no choice but to seek alternatives to antibiotics that can prevent or treat bacterial infections, and especially healthcare-associated infections (Falconer and Brown, 2009; Gorski et al., 2009).

Phage therapy, or more precisely, therapeutic use of lytic bacteriophages to treat pathogenic bacterial infections, is one approach that has great potential as a solution to the serious worldwide problem of drug-resistant bacteria (Parisien et al., 2008; Sulakvelidze, 2005). Discovered by D'Herell in 1915, bacteriophages or phages are viruses that attack bacteria (Sulakvelidze et al., 2001). While they were administered as antibacterial agents as early as 1919, before the discovery of antibiotics, inadequate understanding of phage biology and genetics reduced the efficacy of phage therapy (Gill and Hyman, 2009; Housby and Mann, 2009; Merril et al., 2003). For instance, to remove live bacteria from phage preparations, addition of

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preservatives like phenol or warming the preparations was required, which likely resulted in denaturation and inactivation of phages (Carlton, 1999).

The particulate existence of phages was finally confirmed by electron microscopy in 1940 (Summers, 2001), followed by the introduction of antibiotics. The latter development diverted research attention from phages to the discovery of new antibiotics (Fischetti et al., 2006). Many reviews have described the fascinating history of lytic phage therapy and the reasons that led researchers to reexamine these ancient and ubiquitous magic bullets (Debattista, 2004; Fischetti et al., 2006; Kropinski, 2006; Skurnik and Strauch, 2006). Therefore, we will not discuss the history of phage therapy or its rediscovery in recent years here. Rather, in this review, we will elucidate the increasing emphasis on the rational design of modified phages and what prompted researchers to investigate phage modification.

Although early experiments were performed using lytic phages, interest has shifted to engineered, and mostly non-lytic modified phages (Hagens and Blasi, 2003). Genetically engineered and well-characterized filamentous phages, as well as lytic phages altered by microbial and biological methods, have emerged as alternatives. A majority of studies based on these modified phages have yielded promising data regarding their efficacy. There is special interest in modifying filamentous phages, because they are easy to manipulate genetically. Advances in the genetic and chemical engineering of filamentous phages have facilitated their therapeutic application (Yacoby and Benhar, 2008). However, these advances were mostly limited to *E. coli* phages. Due to high host specificity, suitable phages will need to be developed for each species. Whereas lytic phages and modified filamentous phages release their progeny into the surrounding media, non-replicating modified phages render the entire technique safer (Westwater et al., 2003).

2. Why modified phages?

Efficacy of natural phages against antibiotic-resistant *Staphylococci* (O'Flaherty et al., 2005), *Streptococci*, *Escherichia*, *Pseudomonas*, *Proteus*, *Salmonella*, *Shigella*, *Serratia*, *Klebsiella* (Kumari et al., 2010), *Enterobacter*, *Campylobacter*, *Yersinia*, *Acinetobacter* and *Brucella* are being evaluated by researchers (Matsuzaki et al., 2005). However, in the last few years, modified phages are increasingly being explored, mostly due to the limitations of phage therapy using lytic phages. The undesirable side-effects of phage therapy using lytic phages, safety concerns regarding spontaneously propagating live microorganisms and the inconsistency of phage therapy results in the treatment of bacterial infections specifically induced scientists to explore more controllable phages (Krylov, 2001). Directed mutation of the phage genome, recombination of phage genomes, artificial selection of phages in vivo, chimeric phages and other rational designs have conferred new properties on phages, including greater therapeutic potential. These new modified phages have been shown to successfully overcome challenges to earlier phage therapy, such as efficacy and safety issues (Skurnik et al., 2007).

2.1. Strategies for enhancing phage lethality

Phage modification strategies often aim to construct lethal phages or to enhance the lethality of current phages to successfully kill antibiotic-resistant bacteria. Thanks to progress in the field of synthetic biology, these modified phages control bacteria more efficiently and may also be used with antibiotics as a combination biological–chemical treatment to reduce the chance of developing residence (Weber and Fussenegger, 2009). In other words, engineered phages act like a strong adjuvant for antibiotic therapy. In one experiment, targeting the SOS DNA repair system using engineered M13mp18 phages, along with antibiotic treatment, increased the killing of antibiotic-resistant *Escherichia coli*, persister cells and even biofilm cells (Lu and Collins, 2009a; Lu and Collins, 2009b). The phage platform established in this study may also be used to target other bacterial specific gene networks. In a similar report, an enzymatic phage was designed to reduce the number of bacterial biofilm cells, which are crucial to the pathogenesis of many clinically relevant infections due to their resistance to antimicrobial treatment and the host immune response. Engineered enzymatic phage was able to express a biofilm-degrading enzyme during infection and consequently could attack the bacterial cells in the biofilm and the biofilm matrix (Lu and Collins, 2007).

In addition to targeting biofilm matrix, phages may be used to damage the bacterial cell wall. This damage allows antibiotics to pass through the outer membrane of Gram-negative bacteria, permitting access to their site of activity despite the drug hydrophobicity and large size. As in the case of combining β-lactam antibiotics with β-lactamase inhibitors, which prevent antibiotic degradation by bacteria, a filamentous phage-based strategy reduces the required effective dose of antibiotics. Therefore, the toxicity of high doses of antibiotics, such as in the form of bacterial imbalance or dysbiosis, is reduced. Specifically, in filamentous phage therapy, point mutations in the genes responsible for phage progeny extrusion increase damage to the bacterial outer membrane, enhancing antibiotic vulnerability. Subsequent development of drug resistance and other unwanted side effects are thus likely to be reduced (Hagens et al., 2006). In another form of combination therapy, one research group demonstrated that filamentous phages may be used as universal drug carriers. They engineered a phage to display a specific moiety linked to chloramphenicol, a model drug. The filamentous phage, as targeted drug carrier, selectively attacks *Staphylococcus aureus* cells (Yacoby et al., 2006).

There have been attempts to increase the efficacy of phages, particularly by altering the phage environment rather than the structure or genome. Addition of free endosialidase to a culture of the *E. coli* bacterial host of serotype 018:K1:H7, with the aim of increasing the penetration of the K1-ind phage, is a good example of one of these attempts. After Smith and Huggins revealed that the type of phage used in phage therapy is substantially important (Smith and Huggins, 1982), Bull at al. determined that the endosialidase activity of the tail spike is necessary for infection of capsulated cells in serum. This may explain why the phages employed by Smith and Huggins to treat infection did not perform equally. Such modifications to the phage–bacteria environment thus may improve treatment success, even though they do not need a genetic modification (Bull et al., 2010).

2.2. Narrow host range of lytic phages and host specificity

Antibiotic treatment can lead to serious secondary infections involving relatively resistant bacteria, which may increase hospitalization time, expense and mortality, especially in the case of *Pseudomonas* (Niederman, 2001) and *Clostridium difficile* (Kyne et al., 2002; Pepin et al., 2005). Phages can be targeted far more specifically than most antibiotics to particular bacteria, resulting in much less damage to the body's normal microbial balance. Although genome rearrangements and mutations in specific genes, such as that encoding endolysin, can extend the host range of phages, phages are basically species-specific antibacterial agents (Kasparek et al., 2007). Indeed, the host specificity of phages is extremely refined, with each phage only invading one species or even a single bacterial strain. Therefore, a broad-host-range phage is of paramount importance for therapeutic application (Knezevic et al., 2009).

Historically, incorrect phage selection and utilization of one type of phage to treat infections caused by mixtures of different bacteria resulted in misleading data regarding phage therapy. To avoid failure in phage therapy resulting from narrow spectrum of phage host specificity, either an accurate diagnosis must be obtained prior to

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