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Prospects to Enhance Phage Therapy by Looking At CRISP Fingerprints in Bacterial Populations

Vladimir Zlobin I[1]*, Yuri Dzhioev P[1], Nadezhda Peretolchina P[1], Andrey Borisenko Y[1], Liliya Stepanenko A[1], Yingchen Wang[2], Zhangyi Qu[2], Rian Pierneef[3] and Oleg Reva N[3]

[1]*Scientific and Research Institute of Biomedical Technologies of the Irkutsk State Medical University, Russia*

[2]*Department Microbiology, Harbin Medical University, 157, China*

[3]*Department of Biochemistry, University of Pretoria, South Africa*

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***Corresponding author:** Vladimir Zlobin I, Scientific and Research Institute of Biomedical Technologies of the Irkutsk State Medical University, Irkutsk, Russia, Tel: 7-914-926-2451; Fax: 7 395 224-38-43; Email: vizlobin@mail.ru

Abstract

In the last decade CRISPR/Cas systems have been used extensively in different areas of research from bacterial genotyping to genetic engineering. CRISPR/Cas systems are accurate and precise molecular devices designed by the nature, with the primary role being bacterial defence against foreign genetic elements, mainly bacteriophages. The recent advances in sequencing technologies have created opportunities for application of CRISPR/Cas systems in personalized phage therapy and in fundamental studies of co-functioning of bacterial and phage populations in the nature. However, as always, new opportunities mean new technical challenges and demands for innovative bioinformatics tools. This review introduces an initiative of the BRICS consortium, consisting of three research centres in Russia, China and South Africa, to develop a comprehensive interactive resource to source data and software tools for personalised phage therapy.

Keywords: CRISPR/Cas system; Phage therapy; Metagenomics; Bioinformatics

Abbreviations: CRISPR: Clustered Regularly Interspaced Short Palindromicrepeats

Introduction

Initially it was thought that prokaryotes were defenceless against bacteriophages. The first discovery arguing this opinion was the detection of specific bacterial restriction enzymes cutting foreign DNA molecules at specific sites [1]. Then several more mechanisms of bacterial defence against phages were discovered, which included adsorption resistance involving a reduction of affinity of viral particles to the target receptors; and abortive mechanisms of apoptosis of infected bacterial cells [2]. In comparison to the above mentioned mechanisms, CRISPR/Cas systems are the most sophisticated mechanisms of the adaptive immune system to provide defence against bacteriophages [3]. Recently, bacterial CRISPR/Cas systems displayed huge practical significance, especially in the medicinal field [4]. First, these systems are important for prediction of bacterial resistance to phages and for vaccination of industrial bacteria against bacteriophages and plasmids. Second, CRISPR arrays may be used for strain identification due to uniqueness of spacer regions in each strain. Multiple CRISPR/Cas systems of three major types and various sub-types were identified in sequenced bacterial genomes [5]. Advances in sequencing technologies made it possible to investigate the real variety of bacteriophages and CRISPR/Cas elements in nature through the prism of metagenomic sequencing of environmental DNA samples [6]. This new opportunity allows revising approaches of optimization of personalized phage therapy to fight bacterial infections, mainly multidrug resistant infection, by assessing the efficacy of different phage races by inspecting the diversity of the CRISPR/Cas elements in populations of pathogens.

Discussion

CRISPR/Cas systems integrate DNA fragments of phages and plasmids in specific bacterial genome sites. These lociare transcribed into pre-crRNA under control of the bacterial quorum sensing factors [7]. Matured crRNA create complexes with the Cas-proteins, which cleaves foreign nucleic acid at complementary sites recognized by crRNP [8]. The CRISPR/Cas system may be applied in a variety of routes for inhibiting pathogenic microbes. A prospective approach consists in the introduction of antisense DNA sequences into phage vectors that specifically inhibit growth of pathogenic bacteria [9]. Such an effect can be achieved by specific degradation of dsRNA by a selected CRISPR-system. An alternative approach consists in the creation of vector constructions harbouring target genes

and repeats that will be recognized by the CRISPR complex. In this case, the production of phages will be more effective due to avoiding of the suppression of phage genes by the CRISPR/Cas system. As a result, new engineered phages can be created to combat the target infection [10-12]. It is known that pathogenic bacteria acquire resistance to antibiotics at an alarming rate. Presently, about 700 thousand humans are dying from infections caused by resistant bacteria. WHO stated that humanity has entered into the post-antibiotic era, when many common infections became fatal. Phage therapy is considered as a prospective way to reduce the unnecessary use of antibiotics and slow down the drug resistance development [13]. However, to make phage therapy effective and sustainable, the problem of development of phage resistance in pathogens by acquisition of CRISPR/Cas systems has to be overcome. It is accepted that our understanding of the dynamics of circulation of the CRISPR/Cas elements in bacterial populations is in its infancy. Until recently sequences of CRISPR/Cas elements were identified predominantly in complete genomes of cultured bacterial strains. In total 8,939 CRISPR sequences identified in 3,261 prokaryotic and archaeal organisms out of 7,014 tested genomes were recorded in the CRISPRs database [14]. More than 50% of genomes are free from CRISPR/Cas systems, whereas others possess several non-identical copies of these elements per genome. It remains unclear if the presence or absence of the CRISPR/Cas elements is a characteristic of a bacterial population or an individual isolate? Application of metagenomics for reconstruction of various CRISPR loci in bacterial population revealed a much greater variety of these systems than was reported for culture based isolates [15]. In the latter publication it was reported that the turnover rate of the spacer regions in the populations is rather high. The oldest spacers located at the end of the CRISPR repeat regions usually keep functioning for an average of 5 years before losing their efficacy due to mutations in the spacers and in the target phage sequences that presumably is followed by an excision of these regions from the CRISPR element or by a degeneration of whole CRISPR/Cas elements [16]. Such high turnover rates for CRISPR elements is necessary for maintaining the high level of anti-phage immunity in populations, but this discovery calls into doubt previous reports on an effective use of CRISPR sequences for genetic typing of bacterial clades and lineages, which would only suffice if the reported marker CRISPRs were inactive and thus remained immutable.

Conclusion

Recent discoveries of unprecedented and dynamic variability of the CRISPR/Cas systems in bacterial populations indicated a need to development more sophisticated computational systems for interaction modelling between the populations of phages and target pathogens, as well as the creation of more comprehensive resources coupling together the variety of spacer sequences of CRISPR/Cas systems to the abundance of target sequences in phages. Co-infection models by several viruses were considered [17], while in another paper an application of broad host viruses was advocated for effective phage therapy [18]. A mathematical model of phage interaction with CRISPR-immune bacteria [19] and a number of new computational tools for detection of CRISPR elements in metagenomes [https://omictools.com/crispr-detection2-category] have been published. Recent advances in synthetic biology may narrow the gap between a phage sequence database and personalized phage preparation in clinical settings, including whole genome synthesis of bacterial phages in Escherichia coli [20]. Cell free systems may serve as an alternative method for the production of phages for personalized medicine in a completely chemically synthesized manner [21]. More efforts are needed to consolidate available tools and databases to ensure an effective selection of phage strains and combinations thereof for use in personalised medicine.

Conflict of Interests

No conflicts of interests were reported by the authors.

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