Broad Host Range Bacteriophage For Reduction Of Klebsiella Pneumoniae As Potential Therapy In Primary Sclerosing Cholangitis (Psc)

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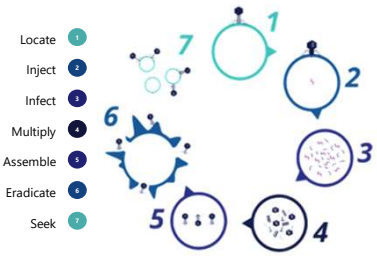
Abstract

Primary sclerosing cholangitis (PSC) is a rare immune-related disease. Strains of *Klebsiella pneumoniae* (*KP*) have been suggested as a potential therapeutic target in PSC, as *KP* isolated from PSC patients' stool were shown to cause Th17 immune stimulation and an increase in gut permeability in germ-free WT or DDC-treated mice (Nakamoto, 2019). With the current understanding of the limitations and undesired effects of antibiotics and the increased prevalence of antibiotic resistant *KP* strains, bacteriophage ('phage') therapy offers a promising treatment to target pathogenic *KP* that are associated with PSC, while leaving the rest of the microbiome untouched. The aim of the current study was to develop a broad-range phage cocktail that can target *KP* species.

Background - phages

Phages are naturally occurring viruses that kill specific bacteria. Unlike antibiotics, phages are specific to the strain level and therefore have unique advantages in terms of minimizing perturbation of the microbiome. They have no capability to infect mammalian cells and so are considered safe. Phages are also unique in that they are self-amplifying and, once their target bacteria is eliminated, they are passively cleared of the clinical system. Combinations of phages are utilized to prevent the appearance of resistant mutant bacteria.

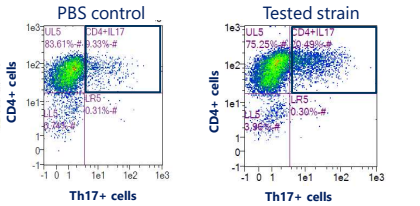
Phages are self-amplifying therapeutic agents



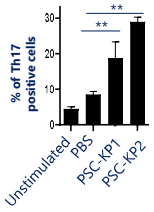
Results

1. *K. pneumoniae* clinical isolates induce a pro-inflammatory Th17 response

Selected clinical strains isolated from PSC patients were tested for their ability to induce a Th17 response in co-cultures of naïve CD4 and CD11c cells



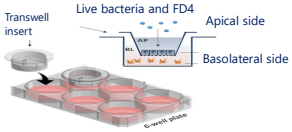
FACS analysis demonstrated a significant increase in the Th17+CD4+ cell population upon exposure to different clinical KP isolates). (**p<0.01, 9% PBS baseline control vs. 20%-30%



2. *K. pneumoniae* clinical isolates induce epithelial permeability

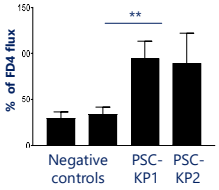
Selected clinical strains were tested for their ability to induce epithelial permeability using a Caco2 cell assay. Live bacteria were introduced on the apical side of a confluent epithelial cell layer together with the small labeling molecule FD4 (FITC-Dextran 4kDa). The presence of FD4 was measured 24h later in the basolateral side.

Study design



Results

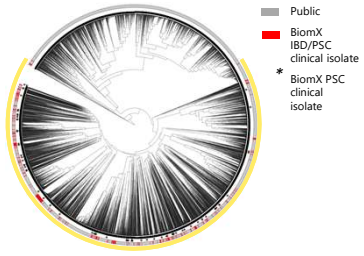
Significant accumulation of FD4 in the basolateral side was induced by *KP* strains (**p<0.01)



3. *K. pneumoniae* clinical isolates: characterization

More than 1,000 KP strains were isolated from 47 PSC patients, 287 IBD patients and 18 healthy subjects. Genome analyses of isolates revealed over 203 unique MLSTs, and 93 unique capsule types

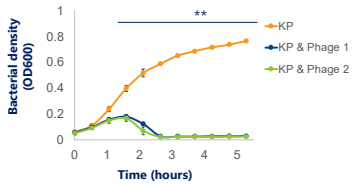
Phylogenetic tree of *K. pneumoniae* strains



IBD and PSC isolates (yellow line) were spread throughout the entire *KP* tree and were not clustered to specific MLSTs.

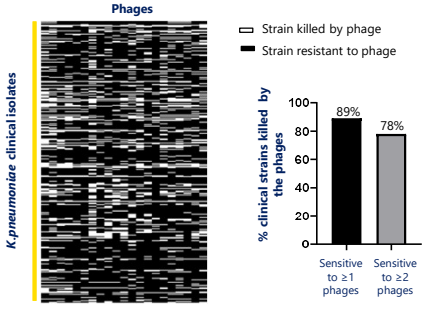
4. Phage isolation results

Proprietary phage hunting capabilities using a library of environmental sources were utilized to isolate a panel of 29 phages that target a broad range of *KP* strains. Sequence analysis revealed that the phages belong to multiple, diverse genera in the Caudovirales order, containing linear dsDNA genomes (~40-180 Kbp). Further analysis confirmed that the identified phages are devoid of undesirable genes as stipulated by regulatory authorities. To test the phages ability to efficiently reduce isolates burden *in vitro,* more than 16,000 infection efficiency tests were conducted. Below is an example, demonstrating high potency of two phages in eliminating bacteria grown in liquid culture (p<0.01 **).



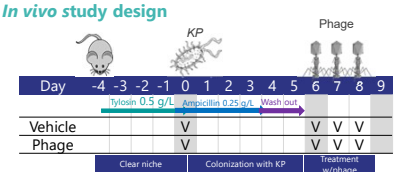
5. Development of a phage cocktail to eradicate *K. pneumoniae*

High throughput infection assays showed that 89% of tested clinical strains could be eradicated by the phages identified. 78% of the isolates were sensitive to more than a single phage. The optimal phages will be selected to design a cocktail of up to 12 phages that targets over 85% of strains.



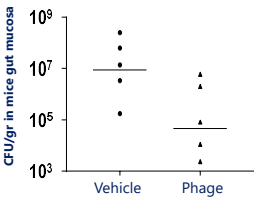
6. Broad host range phage efficiency *in vivo*

***In vivo* study design**



Results

Example of oral administration of a single phage. Reduced target bacterial load by 2 logs is observed in the gut mucosa of colonized animals.



Discussion and Conclusions

The functional evidence for the association of *K.pneumoniae* with causation and progression of inflammation, in IBD and PSC, have been previously demonstrated[1,2] and are supported by our findings. To develop a broad phage cocktail targeting the relevant strains of *K.pneumoniae,* we first isolated an extensive range of strains from PSC and IBD patients. We then applied our phage discovery platform to identify a set of phages that collectively eradicate the majority of relevant *K. pneumoniae* strains. Jointly, the results demonstrate the ability to find a broad host range phage cocktail targeting diverse *KP* species, which may provide a novel treatment approach for PSC patients.

Reference

1. Nakamoto et al. 2019, *Nature microbiology*
2. Atarashi et al. 2017, *Science*

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