Bacteriophage Communities in Osteoarthritic Joints

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INTRODUCTION: Osteoarthritis (OA) is a multifactorial joint disease characterized by cartilage degeneration and systemic inflammation. The emerging "gut-joint axis" concept highlights the role of microbiome dysbiosis in OA pathogenesis. While bacterial communities have been extensively studied, bacteriophages—viruses that infect bacteria—remain understudied despite their potential to modulate bacterial populations and influence disease outcomes. This study aimed to characterize bacteriophage diversity and functional profiles across multiple anatomical sites in OA patients using shotgun metagenomic sequencing.

METHODS: This study was approved by the institutional review board with informed consent obtained from all participants. Oral swabs, fecal swabs, and synovial tissue samples were collected from 9 patients diagnosed with osteoarthritis. DNA was extracted using standard protocols, and sequencing libraries were prepared using IDT xGen NGS Library Preparation kit. Shotgun metagenomic sequencing was performed on Illumina NextSeq 2000 platform. Human reads were depleted using Bowtie 2 alignment against GRCh38 genome. Remaining reads underwent de novo assembly using MetaSPAdes. Viral sequences were identified using VirSorter, with taxonomic classification performed using DIAMOND alignment against the Gut Phage Database. Functional analysis was conducted using EggNOG, and viral lifestyle predictions were performed using pharokka 1.7.5 and Unified Human Gut Virome catalog.

RESULTS: Analysis revealed diverse bacteriophage communities with low taxonomic classification success (15.7%), indicating substantial novel phage diversity. Temperate phages dominated all sample sites but showed progressive enrichment from oral (70.9%) to fecal (75.7%) to synovial tissue (93.4%), with synovial samples containing only 6.6% obligatory lytic phages. Synovial phages exhibited significantly different functional profiles compared to oral and fecal counterparts ($p < 0.001$). Transcription (COG K) and nucleotide metabolism (COG F) genes were completely absent in synovial tissue but present in other sites. Conversely, replication machinery (COG L) was most abundant in synovial tissue (21.62) versus fecal (14.24) and oral samples (10.87). Unknown functions (COG S) showed progressive enrichment from fecal (23.89%) to oral (27.07%) to synovial tissue (32.43%).

DISCUSSION: The identification of diverse bacteriophage communities within synovial tissue from osteoarthritis patients represents a significant advancement in understanding the gut-joint axis and its viral components. The demonstration of bacteriophages targeting pathogenic bacteria—including Acinetobacter, Klebsiella pneumoniae, and Pseudomonas—within arthritic joints provides compelling evidence for viral-mediated bacterial modulation in joint pathology. These findings complement recent investigations by Chen and colleagues, who demonstrated that OA patients exhibit significantly different gut viral communities compared to healthy controls, with 157 disease-associated viral operational taxonomic units achieving remarkable diagnostic accuracy. Our results suggest that phage dysbiosis extends beyond the gastrointestinal tract to affect joint tissues directly, further supporting the emerging gut-joint axis concept. The predominance of temperate phages (93.4%) in synovial tissue suggests lysogenic integration supports stable phage-host relationships within inflamed joints rather than active bacterial lysis, potentially contributing to chronic inflammatory states. The tissue-specific functional adaptations, particularly the complete absence of transcription and nucleotide metabolism genes combined with enhanced replication machinery in synovial phages, indicate specialized survival strategies within the inflammatory joint microenvironment. The substantial proportion of unknown functions (32.43%) in synovial phages highlights the novel nature of these viral communities and suggests untapped therapeutic potential. Recent systematic reviews have confirmed the gut microbiome's role in OA pathogenesis, with dysbiosis characterized by increased Firmicutes/Bacteroidetes ratios and elevated lipopolysaccharide levels contributing to synovitis and joint degeneration. The therapeutic implications are particularly noteworthy given documented success of phage therapy against multidrug-resistant bacterial infections in joint tissues. Study limitations include small sample size (n=9), cross-sectional design preventing temporal assessment, and the exploratory nature requiring validation in larger cohorts.

SIGNIFICANCE/CLINICAL RELEVANCE: This study provides first evidence of tissue-specific bacteriophage communities in osteoarthritis, revealing substantial novel viral diversity and functional specialization within synovial tissue. These findings expand understanding of the gut-joint axis to include viral components and open new therapeutic avenues for leveraging naturally occurring or engineered phage communities to restore microbial balance and reduce joint inflammation in osteoarthritis patients.