

The Colon Cancer Virome and Its Interactions with the Microbiome

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Introduction

Colorectal cancer is a common and severe disease that is the second leading cause of cancer-related deaths in the United States. The US National Cancer Institute estimates over 1.5 million Americans will be diagnosed with colorectal cancer in 2016, and over 500,000 Americans will have died from the disease¹.

Although the cause of colorectal cancer remains unclear, its occurrence has been strongly associated with gut bacterial communities. This association is so striking that bacterial community signatures have been leveraged as biomarkers to greatly improve colorectal cancer detection^{2,3}. While an understanding of colorectal cancer bacterial communities have proven fruitful, other microbial groups have yet to be evaluated and may provide additional insights into the link between disease and microbial communities.

Due to their mutagenic abilities and their general propensity for functional manipulation, viruses are strongly associated with, and in many cases cause cancer. Unfortunately there is not much research

Results

The colorectal cancer virome cohort

Stool samples were collected in accordance with our approved IRB protocol. The cohort consisted of 90 human subjects, 30 of which served as healthy controls, 30 that had adenoma lesions consistent with the pre-cancerous state, and 30 that had carcinoma lesions consistent with colorectal cancer (**Figure 1**). Half of the stool was aliquoted and used to sequence the bacterial communities using both 16S rRNA and shotgun sequencing techniques. The 16S rRNA sequences were reported in a previous publication². The other half of the stool samples were purified for virus like particles (VLPs) and virome genomic DNA extraction, followed by shotgun metagenomic sequencing. The virus purification allows us to observe the *active virome* because we are only sequencing those viruses that were encapsulated (we do not detect viruses integrated in bacterial genomes).

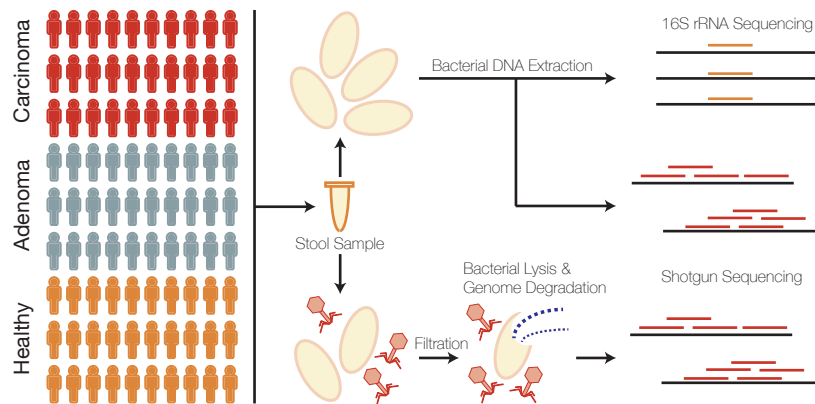


Figure 1: Cohort and sample processing outline. Thirty subject stool samples were collected from healthy, adenoma (precancer), and carcinoma (cancer) patients. Stool samples were split into two aliquots, the first of which was used for bacterial sequencing and the second which was used for virus sequencing. Bacterial sequencing was done using both 16S rRNA amplicon and whole metagenomic shotgun sequencing techniques. Virus samples were purified for viruses using filtration and a combination of chloroform (bacterial lysis) and DNase (exposed genomic DNA degradation). The resulting encapsulated virus DNA was sequenced using whole metagenomic shotgun sequencing.

Virus DNA was purified according to previous studies. Briefly, the stool was resuspended in saline magnesium buffer by rigorous vortexing. Bacteria, human, and other non-viral cells were removed by filtering through a

0.22 μ m filter, followed by cell lysis with chloroform and degradation of the released genomic DNA with DNase (**Figure 1**). The resulting genomic DNA was used to prepare a sequencing library with the NexteraXT preparation kit, and was sequenced on the Illumina HiSeq2500 platform. To accomodate the low concentration of input DNA, we used the NexteraXT protocol with 18 PCR cycles instead of 12. Each run was performed with a blank control to detect any contaminants from reagents. Only one of the controls detected DNA, which was of a minimal concentration, indicating successful sequencing of VLP genomic DNA over potential contaminants (**Figure 2**).

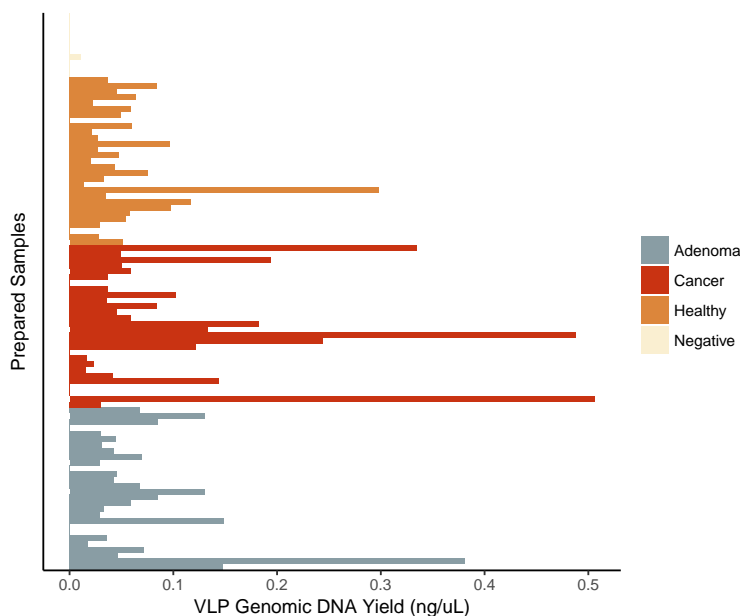


Figure 2: VLP genomic DNA yield from all sequenced samples. Each bar represents a sample which is grouped and colored by its associated disease group.

Cancer-associated virome signatures

The core colorectal cancer virome and auxillary metabolic genes

The colorectal cancer phage-bacteria network

Cancer classification using virus-based machine learning

Previous work has shown that 16S rRNA community signatures are effective for classifying stool samples as coming from healthy, pre-cancerous, or cancerous individuals^{2,3}. This is valuable because it presents a potential alternative screening approach to the invasive colonoscopy. This approach supplements other screening tests such as FIT. The exceptional performance of bacterial signatures in these predictive models suggests a role for bacteria in colorectal cancer. We built off of these findings by evaluating the ability of virus community signatures to classify stool samples and compared performance to models built using bacterial data.

We built and tested random forest models based on virus and bacteria communitiy signatures. To improve performance and make the models computationally feasible, we only used OTUs and OGUs that were present in more than half of the samples. Abundance counts were subsampled to the same level and those samples that failed to reach the defined sequencing threshold were excluded from analysis. The same model approach was used for both datasets, and the only difference was the data used to learn the model. We found that the

model based on virus metagenomic signatures greatly outperformed the model built on bacterial 16S data (**Figure 1**). This supports the utility of using virus communities as cancer biomarkers and suggests viruses play a role in colorectal cancer.

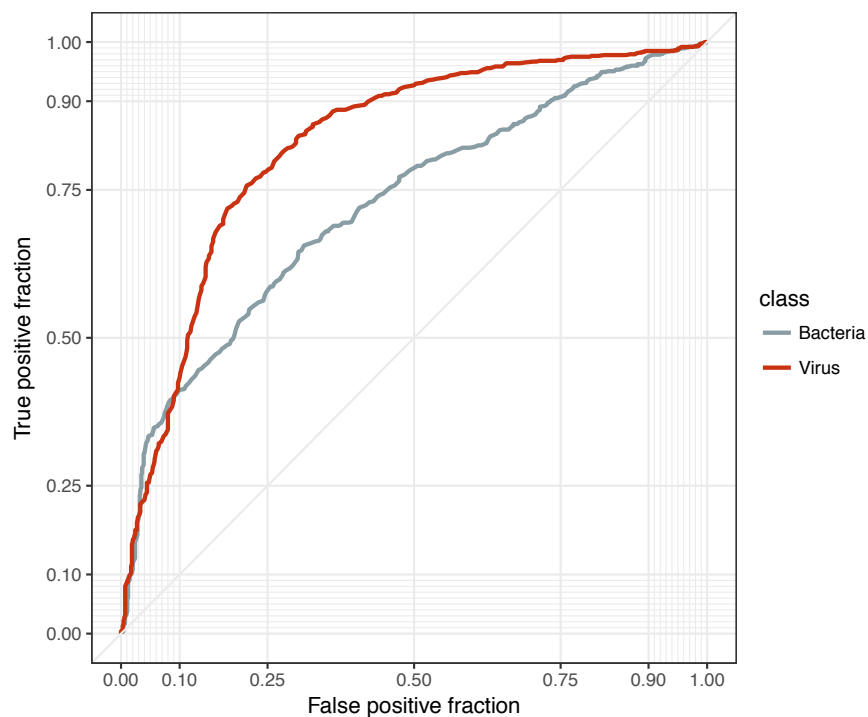


Figure 3: ROC curve from a random forest model built on virus (red) and bacteria (grey) community abundance data.

Discussion

Conclusions

Methods

Acknowledgements

Conflicts of Interest

Figures

References

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