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RESEARCH PROJECT: Decoding the role of DNA methylation in HIV pathogenesis

Background and Rationale

For decades, the human immunodeficiency virus (HIV) has been a major public health concern. Approximately 38 million people are currently living with HIV/AIDS worldwide. To effectively reduce HIV-associated mortality and morbidity, antiretroviral (ARV) treatment consisting of a combination of several drugs is administered. ARVs work by suppressing viral replication; however, a cure is never achieved because the virus persists in a latent state in some cells. Consequently, patients need to take ARVs for the rest of their life which can create difficulties with adherence, drug resistance, and the development of drug-related toxicities over time.

Antiviral therapies targeting host genes that the virus depends on, rather than targeting the virus itself, are promising because these genes do not mutate as frequently as viruses do, and will avoid the development of drug resistance. We must advance our understanding of all factors contributing to HIV infection. While most studies have focused on the influence of host genetic factors on HIV pathogenesis, epigenetic factors are gaining attention. DNA methylation of cytosine nucleotide bases that precede guanine base (CpG) is an epigenetic modification that regulates gene expression. DNA methylation within the promoter and enhancer region prevents the binding of transcription factors, suppressing gene expression. Altered DNA methylation across the host and viral genome has been shown to contribute to HIV disease. Several studies focused on variations in global DNA methylation among uninfected and infected individuals; however, these studies failed to consider the genetic and environmental factors that may influence DNA methylation. Thus, an objective of the study is to assess whole-genome methylation profiles across different stages of HIV infection, (pre-, acute- and chronic- infection) within the same individual. By assessing the methylation profiles of the same individual before, and throughout their HIV infection; we will account for the genetic factors and some environmental factors that may influence DNA methylation. Through this analysis, we will gain a better understanding of the epigenetic regulation of host genes during HIV infection. The identification of differentially methylated host genes will provide novel biomarkers and therapeutic strategies to identify and combat HIV infection.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated protein 9 (Cas9) is a genomic editing tool adapted from the immune defenses of bacteria against invading viruses.

CRISPR-Cas9 editing of the host genome has also been investigated as an intervention against HIV. Several studies have shown that the silencing of human HIV co-receptors, CCR5, and CXCR4 genes, by CRISPR successfully disrupts co-receptors and prevents viral entry, thereby restricting HIV-1 infection. However, one of the main drawbacks of conventional CRISPR is that it permanently switches off genes, and may have undesirable consequences such as off-target gene mutations. Previous studies have shown that the expression of the host genes, CCR5 (responsible for viral entry), BST2 (prevents viral budding), and HLA-A (associated with poor HIV control), are influenced by DNA methylation during HIV infection. Thus, by manipulating DNA methylation at specific CpG sites using CRISPR, we will be able to influence the expression of these genes and thus, control HIV infection. This study aims to identify the influence of DNA methylation on host genes regulating HIV pathogenesis

Objectives and methodology

Objectives: (1) to assess whole-genome methylation profiles across different stages of HIV infection (pre-, acute and chronic infection). (2) To validate the differential methylation status of host genes obtained from the whole-genome methylation analysis. (3) To determine if CRISPR-based manipulation of the DNA methylation status of specific HIV-associated host genes (identified by the genome-wide methylation array and previous studies) can effectively control HIV replication in vitro.

Research Methodology

Genome-wide methylation analysis: Whole-genome methylation will be assessed of 100 individuals followed up over four-time points (pre-, acute-, chronic HIV infection, and post-ARV treatment) using Infinium MethylationEPIC BeadChip (Illumina), which quantitatively profiles 850,000 methylation sites across the entire genome.

Validation of whole-genome methylation analysis: The top ten hits across the different time points identified using whole-genome methylation analysis will be validated using mass array and RT-qPCR. Correlation analysis will be conducted to determine the strength of the gene methylation status and gene expression.

CRISPR/Cas9 targeting of DNA methylation: Vector construction – dcas9 fusion protein design and plasmid construction will be adapted as previously described. Briefly, dCas9SunTag vectors will undergo digestion and ligation to DNMT3A (adds methyl marks) or TET (removes methyl marks). Guide RNA (gRNA) will be designed to flank the region where DNA methylation is to be added or removed. In vitro infection assay – Jurkat cells infected with HIV and control cells will undergo transfection using the CRISPR-Cas9 constructs designed to manipulate DNA methylation at specific

CpG sites of specific HIV-associated host genes (identified by the genome-wide methylation array and previous studies). Assessment of HIV viral load – Supernatants from days 2, 4, and 7 of the in vitro infection will be collected and analyzed for p24 (HIV capsid protein) using the HIV1 p24 ELISA Kit (ELISA [Abcam]). DNA methylation analysis – DNA methylation analysis on Jurkat cells will be conducted as described in the validation section. Gene expression analysis – RT-qPCR will be conducted as described in the validation section. Correlation analysis will be used to determine the relationship between DNA methylation, gene expression, and HIV viral load.

a. Timeline

Objective	Timeline
Identification of top hits from genome wide methylation studies	October 2022 – December 2022
Validation of top hits	January 2023 – March 2023
CRISPR/Cas9 targeting of DNA methylation	April 2023 – August 2023

b. Results or preliminary data

Previous studies by our research group have shown that differential methylation of host genes affected HIV disease progression. These genes included CCR5, CXCR5, BST2, and HLA-A. Furthermore, these studies found that the use of the DNA hypomethylation drug, 5'-Aza-CdR, regulated host gene expression and viral replication. Thus far, from our genome-wide methylation analysis we have found that $CD4^{+}$T cell frequencies, HIV viral load, and methylation patterns from acute/chronic infection significantly differ from pre-infection patterns. Methylation patterns seem to return closer to pre-infection patterns during ARV treatment.