Public Abstract

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Title:Probing the viral replication of HCV and XMRV: Biochemical characterization, inhibition kinetics and role of host proteins in viral replication

The studies described in this thesis focus on RNA viruses XMRV and HCV. In Chapter II we focused on Xenotropic Murine leukemia-Related Virus (XMRV), which was discovered as a novel gammaretrovirus with possible roles in prostate cancer (PC) and chronic fatigue syndrome (CFS). However, further studies showed XMRV was not associated with neither PC nor CFS. We characterized the biochemical activity and kinetics of the XMRV reverse transcriptase and discovered key mechanistic differences between XMRV, Moloney murine leukemia virus (MoMLV), and human immunodeficiency virus (HIV-1) reverse transcriptase (RT) enzymes. Using steady and pre-steady state kinetics we demonstrated that XMRV RT is significantly less efficient in DNA synthesis and in unblocking chain-terminated primers. By surface plasmon resonance experiments we observed that XMRV's decreased DNA binding ability was due to a remarkably higher rate of dissociating from DNA. Consistent with these data, XMRV RT has lower processivity when compared to HIV-1 RT, likely the result of XMRV RT's faster dissociation from bound DNA. Transient kinetics of incorporation of a mismatched nucleotide substrate revealed that XMRV RT has higher fidelity than HIV-1 RT.

In addition to characterizing XMRV RT we determined whether agents known to inhibit MoMLV and HIV-1 RT are effective against XMRV. Hence, we identified RNA aptamers that potently inhibit XMRV, but not HIV-1 RT. XMRV RT is highly susceptible to some nucleoside RT inhibitors, including Translocation Defective RT inhibitors, but not to nonnucleoside RT inhibitors, that are potent against HIV-1 RT. We demonstrated that XMRV RT mutants K103R and Q190M, which are equivalent to HIV-1 mutants that are resistant to tenofovir (K65R) and AZT (Q151M), are also resistant to the respective drugs, suggesting that XMRV can acquire resistance to these compounds through the decreased incorporation mechanism reported in HIV-1.

In Chapter III we focused on Hepatitis C Virus (HCV), the causative agent of hepatitis C infection. We studied the role of the Mov10 host factor in the viral replication of HCV. Mov10 is an antiviral host factor that has been reported to restrict replication of retroviruses, including HIV-1. It has also been reported to inhibit HCV. However, the mechanism of this inhibition has yet to be studied. We investigated the effect of Mov10 on HCV infection to determine which steps of the viral life-cycle are affected by overexpression of Mov10. We demonstrate that Mov10 overexpression in human hepatoma cells restricts HCV RNA production from a sub-genomic replicon (genotype 1a) and in a fully infectious virus (genotype 2a) HCV cell culture system. Inhibition of RNA replication in the infectious virus system leads to decreased virus production over time as measured by HCV RNA levels in cell culture media by RT-qPCR, and the viral titer (TCID50/ml) of released virus. In addition to decreasing virus production, overexpression of Mov10 in producer cells decreases the infectivity of the produced virus. In contrast, overexpression of a control P-body protein Dcp1a has no effect on HCV RNA production, virus production, or infectivity of progeny virus.

Confocal imaging of uninfected cells shows endogenous Mov10 to be localized at P-bodies. However, HCV infection results in redistribution of Mov10 to circular structures surrounding lipid droplets where it co-localizes with HCV NS5A and the core protein. Finally, we demonstrate that the RNA-binding function of Mov10 is responsible for its antiviral effect, as Mov10 mutants that affect its helicase or ATP-binding functions have no effect on its antiviral effect, whereas mutations that disrupt the RNA binding ability of Mov10 seem to abrogate its anti-HCV effect. We also show that localization to P-bodies is not required for the antiviral activity of Mov10. Decreasing Mov10 protein expression levels using CRISPR-Cas9 genome editing technology decreased HCV replication and infection levels, consistent with disruption of Mov10-

Ago2/miR122 binding, which would destabilize the HCV genome. Our data reveal a complex balance between Mov10 and HCV, with Mov10 knockdown data suggesting optimum levels of Mov10 are required for HCV infection, whereas Mov10 overexpression is detrimental to the virus.

In Chapter IV we studied several aspects of HCV. (i) We discovered two novel small molecule inhibitors of the HCV helicase that have antiviral function. (ii) We discovered that Dcp2 is a novel HCV host restriction factor that can block HCV replication, and (iii) we provide insights into the mechanism(s) of action of approved and clinically advanced direct-acting antiviral agents (DAAs). Specifically: (i) we screened a chemical library of compounds for inhibitors of NS3's helicase domain (NS3h) and identified two compounds that inhibited NS3h in vitro (~12 μM IC50s). Both compounds were validated as anti-HCV antivirals in cell-based assays. These preliminary hits will be optimized in future structure activity relationship studies.

(ii) Host proteins can restrict viral replication by directly interacting with the affected viruses. However, they may also act indirectly by affecting the interferon (IFN) response pathway. We discovered one such factor, Dcp2. Overexpression of Dcp2 restricts HCV replication (up to 50% decrease) by causing a profound (30-fold) increase in transcription of IFN?. This inhibition of HCV replication leads to a decrease in virus production, but unlike Mov10 it does not affect the infectivity of the virus produced.

(iii) Several DAAs have advanced in clinical trials and have been approved as drugs for the treatment of HCV infection. Among them, NS5A-targeting drugs have been reported to rapidly eliminate HCV RNA. However, their exact mechanism of action is not clear. We and others demonstrate that daclatasvir (DCV) treatment redistributes NS5A in HCV infected cells. Similarly, we show that ledipasvir (LDV) (the recently approved and most potent anti-HCV NS5A DAA), and cyclosporine A (CsA) (that targets the host factor cyclophilin A) also redistribute NS5A into circular structures. We determined that these circular structures were NS5A redistributed at lipid droplets where it co-localized with the core protein. However, the three drugs had distinctly different effects on the size and number of circular structures and lipid droplets per cell. Additionally, LDV treatment disrupted the localization of HCV dsRNA to lipid droplets and its association with NS5A. We provide insights into the mechanism of action of DCV, CsA, and LDV and their effects on viral complexes and processes.

This work provides insights into the replication processes of XMRV and HCV, strategies to restrict them, the mechanisms of action of drugs that inhibit them, and for HCV; its interaction with host restriction factors.