

INVESTIGATION OF THE PROTECTIVE ROLE OF MIR-122 AGAINST CELLULAR SENSORS OF RNA AT THE 5' TERMINUS OF HEPATITIS C VIRUS GENOME

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Background: Approximately 200 million individuals worldwide are infected by hepatitis C virus (HCV). MicroRNA-122 (miR-122) is a highly abundant liver-specific miRNA shown to interact at two miRNA-binding sites in the 5' end of the HCV genome. This unusual interaction promotes HCV RNA accumulation in both HCV-infected cells and the livers of infected patients. Previous investigation of the stabilization of HCV RNA by miR-122 shows a slowed rate of decay in cells supplemented with miR-122 duplexes. Recent findings demonstrate that miR-122 protects HCV RNA from degradation by exoribonucleases. These results support a model whereby miR-122 acts to shield the 5' terminus of the viral RNA, preventing its degradation or recognition by nucleases or cellular sensors of RNA. Protein kinase R (PKR) is activated mainly by long dsRNA, but short RNA stem-loops can activate PKR in a 5' triphosphate-dependent manner, suggesting that the 3' overhang created by miR-122 binding to the HCV 5' end may also prevent recognition of HCV by PKR. In addition, the LGP2 protein is another RIG-I-like receptor that binds to dsRNA and acts as an on/off switch for RIG-I signaling.

Aims: We hypothesize that miR-122 forms a distinct complex with host and/or viral proteins that together protect the HCV 5' terminus from recognition by cellular sensors of RNA, such as PKR and LGP2. Herein, we are investigating a protective role for miR-122 against these cellular sensors of RNA.

Methods: We are inhibiting PKR and LGP2 expression by siRNA knockdown in Huh7.5 cells, in the presence or absence of miR-122. To investigate the stabilization of the viral RNA in this context, we are monitoring viral RNA accumulation by luciferase assay and northern blot analyses. To investigate the contribution of miR-122, we are using miR-122 site mutants or sequestering miR-122 using an antisense locked nucleic acid inhibitor.

Results: We demonstrate that LGP2 expression is increased early during HCV infection in Huh7.5 cells. Knockdown of PKR or LGP2 in the presence of miR-122 has no significant effect on HCV RNA accumulation. Our current focus is on elucidating the effect of PKR and LGP2 knockdown on HCV RNA accumulation in miR-122 site mutants under limited miR-122 conditions or during miR-122 sequestration.

Conclusions: We expect that the results will reveal whether miR-122 binding to the 5' terminus of HCV is protective against recognition by the cellular sensors of RNA, PKR and LGP2 and together with our collaborators in the Wilson lab, we are investigating the role of several other sensors of RNA, including IFIT-1, IFIT-5, RIG-I and MAVS. These results will provide insights into whether miR-122 binding to the HCV genome protects the viral RNA from recognition by cellular sensors of RNA and has implications for the mechanisms of miR-122 mediated

promotion of HCV RNA accumulation.

Funding Agencies: CIHRCanHepC, FRSQ