Study details microRNA's role as a double agent during Hep C infection

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When researchers looked at sites on liver cell genomes where the gene-regulating molecule miRNA-122 binds, they found that infected cells (red) had fewer of the normal interactions with miRNA-122 compared to uninfected cells (blue). This suggests the virus alters gene expression by sponging up miRNA-122. Credit: Zach Veilleux / The Rockefeller University

In the battle between a cell and a virus, either side may resort to subterfuge. Molecular messages, which control the cellular machinery both sides need, are vulnerable to interception or forgery. New research at Rockefeller University has revealed the unique twist on just such a strategy deployed by the liver-infecting Hepatitis C virus - one that may help explain the progression of liver disease and that the researchers

suspect may be found more widely in the world of disease-causing viruses.

Led jointly by Charles Rice, the Maurice R. and Corinne P. Greenberg Professor in Virology and head of the Laboratory of Virology and Infectious Disease and Robert Darnell, Senior Attending Physician, Robert and Harriet Heilbrunn Professor, and head of the Laboratory of Molecular Neuro-oncology, the research is described today (March 12) in *Cell*. It employed a powerful combination of techniques to map the interactions between the virus and a small piece of genetic material - known as miRNA-122 - that is produced almost exclusively by liver cells, which normally use it to regulate expression of their own genes.

"It is well known that once inside a liver cell, the hepatitis C virus must bind to miRNA-122 in order to establish a persistent infection. We found an unanticipated consequence of this interaction: By binding to miRNA-122, the virus acts like a sponge, soaking up these gene-regulating molecules," says first author Joseph Luna, a graduate student with a joint appointment in the labs. "Our experiments showed this has the effect of skewing gene activity in infected liver cells."

The fight between an infecting virus and its host is often viewed as proteins fighting like soldiers. And soldiers on both sides must have orders, in this case the genetic information responsible for the production of proteins, Luna explains. This is where miRNA-122 comes in. It is a microRNA, a type of RNA encoded into the genome for the purpose of turning down the expression of genes. It does this job by guiding a complex of silencing proteins to an RNA transcript of a gene so as to prevent it from being turned into a protein. In this way, miRNA-122 appears to help control a number of normal functions, including cholesterol and iron metabolism, as well as circadian rhythms.

But miRNA-122 is also necessary for hepatitis C virus. Once in a liver

cell, the viral RNA binds with miRNA-122, which stabilizes and protects the virus so it can replicate. Over the long term, the hepatitis C virus infections can lead to scarring of the liver and liver cancer.

In order to explore how the hepatitis C virus and miRNA-122 interact and the repercussions for the host liver cells, the researchers used a technique known as cross-linking and immunoprecipitation (CLIP), developed over the past 12 years by the Darnell lab, which was targeted to find Argonaute, one of the proteins involved in silencing. This way, they captured the miRNA-122/Argonaute complexes and the sections of RNA transcript to which they bound. They then sequenced those RNA transcripts to see what genetic instructions they represented.

"One microRNA can have hundreds of targets, but most often studies are driven by anecdotes focused on single interactions. By combining CLIP and RNA sequencing, however, we were able to take a global perspective and map out all of miRNA-122's interactions across both the viral and infected host genomes," says study author Robert Darnell, who is also a Howard Hughes Medical Institute Investigator. "The result is a rigorously constructed picture of what is actually going on in the cell."

Their maps showed a peak in miRNA-122 binding at one end of the viral genome, confirming previous work; miRNA-122 also interacted with the virus in a number of other places, for which the significance is not yet known. When the researchers looked at miRNA-122 interactions in an infected cell, they found lower levels as compared to uninfected cells. What's more, a look at the expression of genes regulated by miRNA-122 confirmed this microRNA was less active because those genes had higher levels of activity.

"What if chronic low levels of miRNA-122 prompt changes that, over years, contribute to liver damage and cancer? This could be a molecular link between the viral infection and the pathologies associated with

hepatitis C," Luna says. "More work on miRNA-122 targets in hepatitis C-infected patients may clarify why some go on to develop liver cancer and others don't."

The hepatitis C virus isn't the only virus known to alter gene expression in host cells by sponging up their microRNAs. Human cytomegalovirus and herpesvirus saimiri, which infects New World monkeys, employ a similar strategy, producing RNAs specifically to bind up host microRNAs. But, unlike the others, the hepatitis C virus must bind to a microRNA to replicate, and when it does so, the new viral genomes sop up even more miRNA-122, creating a positive feedback.

"We suspect these three cases may just be the tip of the iceberg, that other viruses - perhaps those that replicate much more ferociously - may use similar microRNA-sponging strategies," study author Charles Rice says. "The techniques we used will make it possible to investigate the use of this strategy in an unbiased way."

Provided by Rockefeller University