Hepatitis C Virus Infections, Inflammation and Liver Cancer

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LAY SUMMARY

Inflammation can be beneficial or harmful. Inflammation is beneficial when it eliminates bacterial and viral infections. On the other hand, inflammation can be harmful when it fails to eliminate infections. Inflammation associated with chronic bacterial and viral infections causes cancer. *Helicobacter pylori* infections are the most common cause of stomach cancer. Hepatitis C virus (HCV) and hepatitis B virus (HBV) infections are the most common causes of liver cancer. These infections do not cause cancer directly. Rather, *H. pylori* and hepatitis viruses cause cancer indirectly due to the inflammation associated with chronic infections. The host immune response, which fails to eliminate the infections, produces inflammation nonetheless. Ongoing destruction of host tissues due to inflammation over prolonged periods of time leads to stomach cancer in people with *H. pylori* infections and cirrhosis, liver failure, and liver cancer in people with hepatitis virus infections.

Researchers from UCLA used multiple-cause-of-death data from the US National Center for Health Statistics to document 7,427 HCV-related deaths in 2004 (Wise, 2008): 3,230 due to cirrhosis and fibrosis (43.5% of HCV-related deaths), 2,347 due to liver failure (31.6% of HCV-related deaths), and 1,359 due to primary liver cancer (18.3% of HCV-related deaths). According to the American Cancer Society 18,910 people (12,720 men and 6,190 women) died from liver cancers in the United States in 2010 (www.cancer.org). Researchers from USC used private insurance claims data to measure the financial burdens associated with the initial diagnosis of an HCV infection They found that private insurance claims for a patient with a newly diagnosed HCV infection were $37,390 per year versus $13,575 per year for insured patients of the same age without an HCV infection (McCombs, 2011). A significant portion of these increased insurance claim costs were associated with interferon-based antiviral treatment regimens. Interferon-based antiviral treatments prevent HCV-related deaths if the treatment succeeds. Unfortunately, up to 50% of HCV-infected patients fail to benefit from these expensive therapies because HCV infections are notoriously resistant to interferon-based treatments (Manns, 2001).

We plan to study specific host and viral molecules involved in the inflammatory responses to chronic HCV infections. Dr. Barton's lab discovered the manner in which RNase L detects and destroys HCV RNA. The Barton lab carefully mapped the location and features of RNase L cleavage sites in HCV RNAs and found that HCV RNAs from more interferon treatment-resistant strains have fewer potential RNase L cleavage sites than HCV RNAs from more interferon treatment-sensitive strains, consistent with an important role of RNase L in the outcomes of interferon-based antiviral therapies. In conjunction with other collaborators, Dr. Barton's lab identified a fragment of HCV RNA produced by RNase L that provokes inflammation. In order to study the impact of RNase L on HCV infections in patients, the Barton lab initiated a collaboration with the Hesselberth and Rosen labs. Dr. Hesselberth's lab developed powerful Illumina cDNA sequencing methods to detect and identify RNA fragments like those produced by RNase L. Daphne Cooper, a graduate student in the Barton lab, adapted the method to analyze RNA from virus-infected cells and liver biopsies. Hugo Rosen provides liver biopsies from uninfected and HCV-infected patients, along with expertise in the immune response to HCV infections. Together, this team of scientists has the expertise and resources to elucidate the manner in which RNase L and RNA fragments produced by RNase L influence the balance between beneficial and harmful

inflammation during HCV infections. Currently, the contribution of unusual RNA fragments to liver inflammation and cancer is overlooked and unappreciated.

Rationale, Objectives & Methods: Antiviral endonucleases like RNase L destroy host and viral RNAs at defined sites leaving behind RNA fragments with chemical features not usually present in normal cells (2',3'-cyclic phosphate termini). We developed a novel Illumina cDNA sequencing method to specifically detect and identify RNA fragments with these unusual chemical features. This method easily identifies up to 18 million individual RNA fragments produced by RNase L in each sample for only $875. By including unique barcode sequences in RNA linkers, we can combine multiple cDNA libraries into one Illumina sequencing reaction to achieve cost efficiencies. For instance, cDNA from 5 individual liver biopsies can be combined into one Illumina sequencing reaction, yielding robust data for only $175 per liver biopsy. We intend to use this method to examine the host and viral RNA fragments produced by RNase L in HCV-infected liver biopsies.

Expected Outcomes: We expect to find more abundant amounts of pro-inflammatory RNA fragments in patients with aggressive hepatitis, those prone to develop liver cancer, and those resistant to interferon-based antiviral therapy. These outcomes will further elucidate the root cause of inflammation associated with cirrhosis, liver failure and liver cancer.

References

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