Identifying potential HCV drug leads by small molecule screening.

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Background

Hepatitis C (HCV) is a viral infection of the liver which had been referred to as parenterally transmitted "non A, non B hepatitis" until identification of the causative agent in 1989. About 80% of newly infected patients progress to develop chronic infection. Cirrhosis develops in about 10% to 20% of persons with chronic infection, and liver cancer develops in 1% to 5% of persons with chronic infection over a period of 20 to 30 years.

HCV is a major cause of acute hepatitis and chronic liver disease, including cirrhosis and liver cancer. Globally, an estimated 170 million persons are chronically infected with HCV and 3 to 4 million persons are newly infected each year. HCV is spread primarily by direct contact with human blood.

No vaccine is currently available to prevent hepatitis C. Treatment for chronic hepatitis C is costly and results are unpredictable.

Current research for new treatments for chronic HCV infection, are focused on the immune response or the targets the virus itself. It is therefore interesting to know if variations in hepatic cell function are responsible for the different reactions to infection.
Current treatment

Antiviral drugs such as interferon taken alone or in combination with Ribavirin, can be used for the treatment of patients with chronic hepatitis C, but the cost of treatment is very high. Treatment with interferon alone is effective in about 10% to 20% of patients. Interferon combined with Ribavirin is effective in about 30% to 80% of patients, depending on the genotype of the virus. Ribavirin does not appear to be effective when used alone.

Re-treatment with Interferon combined with Ribavirin in patients having failed to respond to initial course with the same therapy has not been successful, hinting that the difference is genetic rather than environmental. Furthermore the likelihood of cirrhosis in patients with HCV has been linked to a constellation of seven single nucleotide polymorphisms (SNPs), and cirrhosis is by itself a predictor of poor response to treatment.

Material and methods

I propose a method for high throughput screening of a small molecule library for viable treatment options to be used in combination with current therapy (Ribavirin + Peg-interferon), specifically looking for small molecules that alter the mRNA expression profile of non-responders, to mimic that of responders.

It is traditional to biopsy the liver before treatment is initiated so acquiring material should be relatively straightforward. It has been shown that cryopreservation and subsequent inoculation in growth medium for up to 24 hours, has no impact on the gene expression profile of human hepatocytes.

First the gene expression profile of liver cells from responders would be compared to non-responders. The cells would ideally be hepatocytes in suspension, to maximize the yield from each sample. Hepatocytes in suspension only maintain “normal” expression profile for up to 24 hours. This incubation
period is sufficiently long to determine the metabolic stability and to allow identification of the main metabolites of a test substance, but may be too short to allow generation of some minor, particularly phase II metabolites, that contribute less than 3% to total metabolism. To achieve longer incubation periods, hepatocyte culture systems or bioreactors have to be used.

Principal components analysis and hierarchical cluster analysis of the expression profiles are then used to detect major differences between the two groups.

Secondly, if a statistically significant difference is found between the two groups, liver cells from non-responders would be inoculated into growth medium containing one of the small molecules, and then expression profile compared to the controls, looking for the specific changes identified earlier.

Since the typical small molecular library contains around 10,000+ molecules, the experiment is only practical if the samples could be robotically dispensed and analyzed. For example, the Dana-Farber/Harvard Cancer Center uses a small molecule library of over 100,000 compounds and a robotics platform to perform high-throughput screening to identify the effects of small molecules.

**Goal 1: Mapping the genetic difference between responders and non-responders**

The first step is to establish whether a statistically significant difference in the gene expression profile between responders and non-responders can be found.

To increase the homogeneity of the test subjects, only patients infected with one genotype of the hepatitis C virus would be included in the study. In the United State genotype 1 is the most common
strain. Only 45% of patients with genotype 1 achieve sustained viral response with treatment. This low response rate could possible amplify any differences between the groups.

Differences in expression profiles of responders and non-responders will be measured, both in the absence and presence of the current traditional treatment.

Since 20% of those infected with HCV clear the infection spontaneously, it would be interesting to compare their expression profile to the other two groups. Acquisition of liver samples from healthy subjects with previous history of HCV infection is however problematic.

A genealogical survey could also identify possible heredity patterns for HCV infection response.

**Goal 2: High throughput screening of a small molecule library**

If a convincing and consistent difference is found between responders and non-responders, we can continue into phase 2, to screen for viable treatment options.

A plethora of small molecular libraries exists. Different libraries have different strengths. Using small-molecule libraries highly enriched for FDA-approved drugs will possibly provide a more rapid path to clinical application. Another promising set is the DOS set, a library of 8,064 diversity-oriented synthesis (DOS) compounds. This library is a meta-library comprised of DOS libraries from chemists throughout the United States and Canada. Information about the DOS set is available at

References:

- **Richert, L. et al.** Gene expression in human hepatocytes in suspension after isolation is similar to the liver of origin, is not affected by hepatocyte cold storage and cryopreservation, but is strongly changed after hepatocyte plating. Drug Metab. Dispos. 34, 870–879 (2006).