Network based analysis of Hepatitis C Virus infection

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1 Introduction

Hepatitis C Virus (HCV) is a major cause of chronic liver disease in humans infecting about 170 million individuals worldwide. Therefore, there is much interest to understand the biology of HCV infection and develop effective remedies. While, studies have provided some insights into HCV infection such as a critical role for the interactions between the viral component (HCV core protein) and a host regulator PA28\gamma \cite{1}, a detailed understanding of the mechanisms involved in HCV infection remains elusive.

The increasing availability of Protein-protein interaction (PPI) data for different organisms including humans and host-pathogen associations has facilitated the development of network-based models of human diseases. Construction and analysis of such ‘infection networks’ permits a global overview of pathogenesis with the potential to identify novel regulators of cellular infection \cite{2}. To investigate HCV pathogenesis and replication, different biological datatypes were employed for construction of extended ‘infection networks’ and examined for specific functional associations and their significance to HCV pathogenesis.

2 Method and Results

2.1 Generating extended PPI networks

Host proteins that interact with the HCV Core protein (associated with pathogenesis) and NS4B protein (associated with viral replication) were identified using the membrane yeast two-hybrid approach. Similarly, protein arrays were employed to identify a list of differentially expressed proteins from transgenic mice expressing ectopic HCV core protein (CoreTG) with (dataset 1) or without (dataset 2) the silenced endogenous PA28\gamma compared with the wild-type mice (unpublished data); these too were used for network analysis. Two sets of extended Protein-protein interaction (PPI) networks incorporating the secondary interactors of the human proteins interacting with the HCV Core Protein and NS4B and the secondary interactors of the differentially expressed proteins in the transgenic mice were derived with our in house TargetMine data warehouse system (unpublished), which retrieves the interaction data from PPI resources.

2.1 Functional analysis using GO and KEGG annotations and OMIM associations

Gene Ontology term associations and biochemical pathway data were retrieved from the Gene Ontology consortium (GO) \cite{5} and Kyoto Encyclopedia of Genes and Genomes (KEGG) \cite{6} respectively. For each interactor in the extended PPI networks, the enrichment of specific GO terms was estimated using GO Term finder \cite{3} and significantly affected KEGG pathways was estimated with Fisher’s exact test ($p<=0.05$).

2.3 Results
HCV Core protein extended PPI network was enriched in 26 significantly affected KEGG pathways ($p$-value<=$0.05$). These include the Adipocytokine signalling pathway, the disruption of which is strongly correlated with insulin resistance, steatosis and hepatocellular carcinoma (HCC), while its dysregulation leads to poor IFN-$\gamma$ anti-viral therapy [2]. This is consistent with the observed enrichment of T-cell receptor signaling pathway and MAPK signaling pathway. Focal adhesion pathway, which regulates cell migration and deregulation of which is linked with tumour progression and viral propagation, was also enriched in the HCV Core network, providing insights into the role of the Core protein in HCV pathogenesis and viral propagation. The HCV NS4B extended network was significantly enriched in 7 KEGG pathway types, including Complement and coagulation pathway, implicated in immune response and HCV-mediated hepatic cell proliferation and fibrinogenesis and Oxidative phosphorylation, perturbation of which is known to contribute to increased insulin resistance and accelerated fibrosis and steatosis. This suggests novel roles for NS4B in hepatic inflammation, a role hitherto associated with the Core protein.

Functional analysis of the networks constructed for the differentially expressed proteins derived from protein array data was able to highlight the pleiotropic effects of the HCV Core protein. HCV Core protein was found to alter Fatty Acid metabolism and induce Insulin resistance, independently of PA28$\gamma$. Similarly, over expression of the Core protein was sufficient to disrupt focal adhesion and JAK-STAT signaling pathway, invoking IFN-$\alpha$ resistance and a poor anti-viral response. HCV Core protein was also found to perturb the factors associated with SNARE interactions in vesicular transport, consistent with the observed modification of host lipid rafts in HCV replication [4].

3 Discussions

The network analysis helps identify potentially novel regulators that may facilitate HCV infection and viral persistence in the host. The functional information derived here can be used to assist the ranking and scoring of genes based on network properties and proximity to disease genes and to identify several new factors possibly associated with HCV pathogenesis and propagation via Core and NS4B proteins. Preliminary experimental verification of the role of some of these factors in HCV replication and propagation have yielded positive results and further validation may help further understand the involvement of these factors in HCV infection and provide suitable anti-HCV therapeutic targets.

References


