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Exploring the role of human miRNAs in virus–host interactions using systematic overlap analysis

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ABSTRACT

Motivation: Human miRNAs have recently been found to have important roles in viral replication. Understanding the patterns and details of human miRNA interactions during virus–host interactions may help uncover novel antiviral therapies. Based on the abundance of knowledge available regarding protein–protein interactions (PPI), virus–host protein interactions, experimentally validated human miRNA-target pairs and transcriptional regulation of human miRNAs, it is possible to explore the complex regulatory network that exists between viral proteins and human miRNAs at the system level.

Results: By integrating current data regarding the virus–human interactome and human miRNA-target pairs, the overlap between targets of viral proteins and human miRNAs was identified and found to represent topologically important proteins (e.g. hubs or bottlenecks) at the global center of the human PPI network. Viral proteins and human miRNAs were also found to significantly target human PPI pairs. Furthermore, an overlap analysis of virus targets and transcription factors (TFs) of human miRNAs revealed that viral proteins preferentially target human miRNA TFs, representing a new pattern of virus–host interactions. Potential feedback loops formed by viruses, human miRNAs and miRNA TFs were also identified, and these may be exploited by viruses resulting in greater virulence and more effective replication strategies.

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1 INTRODUCTION

miRNAs are key regulators of various biological processes (Bushati and Cohen, 2007). In addition, recent studies have demonstrated that human miRNAs also have diverse roles in mediating virus–human interactions, see survey by Ghosh *et al* (2009). For example, viruses are able to regulate infection-associated host factors by manipulating human miRNAs to facilitate the infection process (Wang *et al*., 2008). miRNAs can also interfere with the virus infection process (Santhakumar *et al*., 2010). By exploring the role of human miRNAs in viral pathogenesis, our understanding of the complex virus–human interactions will be further increased.

Viruses may selectively take advantage of human miRNAs to increase their ability to infect or replicate within hosts. For example, Wang *et al*. found that cytomegalovirus induced changes in miRNA expression (Wang *et al*., 2008). Ho *et al*. also found that enterovirus-induced miR-141 targeted cap-dependent translation initiation factor (eIF4E) resulting in termination of host protein synthesis (Ho *et al*., 2011). Conversely, human miRNAs can also interfere with viral infections. For example, Santhakumar *et al*. found that a subset of human miRNAs regulated signal transduction pathways, including PI3K/AKT and ERK/MAPK signaling pathways, as well as oxidative stress signaling and prostaglandin synthesis. Moreover, these miRNAs were able to confer broad inhibitory potential against multiple viruses (Santhakumar *et al*., 2010). In addition, Lagos *et al*. further confirmed that human miRNAs can regulate innate immune response to viral infections (Lagos *et al*., 2010).

Human miRNAs can also directly target viral genomes. For example, Lecellier *et al*. confirmed that the open reading frame of human foamy virus was targeted by human miR-32, resulting in effective inhibition of human foamy virus replication (Lecellier *et al*., 2005). Similarly, overexpression of human miR-199a inhibited replication of the hepatitis C virus (HCV) genome in cells infected with HCV-1b or HCV-2a replicons (Murakami *et al*., 2009). However, it remains unclear whether human miRNAs that directly target viruses confer resistance to viral infections, or if they are used by viruses to establish infection.

The growing availability of experimental data regarding the human protein interactome, virus–host protein interactions and human miRNA-target pairs has provided a valuable opportunity to study the role of human miRNAs at the systems level. Therefore, in this study, a potential regulatory network was generated using host targets of viral proteins, human miRNAs and putative miRNA TFs. Specifically, four typical patterns of human miRNA-mediated virus–human interactions (Fig. 1), the topological characteristics of host target proteins and human miRNA targets described in the human protein–protein interaction network (PPIN) are included.

2 MATERIALS AND METHODS

2.1 Human protein interaction dataset

The Human Protein Reference Database (HPRD) (Release 9) (Keshava Prasad *et al*., 2009) that overlaps well with other PPI databases was used in this study. The largest connected component with 9270 nodes and 38 855 interactions was obtained by removing small clusters and single nodes. All topological parameters were computed using the largest connected component.

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†The authors wish it to be known that, in their opinion, the first three authors should be regarded as joint First Authors.

2.2 Virus–human interactome dataset

A virus–human protein interaction dataset that included 150 viruses and 3300 interactions was obtained from the VirHostNet database (Navratil *et al.*, 2009). This database collects high quality, up-to-date information curated from public databases.

2.3 Experimentally validated targets and potential human miRNA TFs

By integrating four miRNA target databases, Tarbase (Sethupathy *et al.*, 2006), miR2disease (Jiang *et al.*, 2009), miRecords (Xiao *et al.*, 2009) and miRtarbase (Hsu *et al.*, 2008), an experimentally validated human miRNA target dataset was constructed. This dataset included 3923 miRNA-target pairs. Available information regarding TFs of human miRNAs was downloaded from the PuTmiRdatabase (Bandyopadhyay and Bhattacharyya, 2010).

2.4 Topological analysis of a protein interaction network

The network analyzer plugin for Cytoscape (Assenov *et al.*, 2008) was used to compute the degree and betweenness centrality of proteins in the PPIN. Human proteins in the top 5% of degree and betweenness centrality in the human protein network were defined as hubs and bottlenecks, respectively. *K*-core and excess retention (ER) analyses were also used to measure the distance of the node list from certain properties related to the network center. The *k*-core of a network is defined as the maximum sub-network obtained by pruning all nodes with a degree lower than *k*. The ER value, E_V^A / E^A, is defined as the ratio of the proportion of nodes with property A in the *k*-core, E_V^A, to the proportion of nodes with property A in the original network, E^A.

2.5 Randomization test

To test whether viral proteins and human miRNAs preferentially interact with protein pairs in the human PPIN, human PPIs obtained from the HPRD database (including 39 204 pairs of interactions) were used. First, the actual number of interaction pairs that were targeted by viral proteins and human miRNAs was computed, and then all interaction pairs were shuffled 10 000 times while preserving the interaction number. Each time the number of interaction pairs targeted by viral proteins and human miRNAs was computed (represented as X), the *P*-value was defined as the ratio of the number of X larger than the actual number to 10 000.

To examine the preference for hubs and bottlenecks of the overlapping targets between virus and human miRNAs, the actual proportion of hubs and bottlenecks of the overlapping targets was computed (represented as Y). The same number of nodes (as hubs or bottlenecks) was then sampled from HPRD, and this was repeated 10 000 times. The *P*-value was defined as the ratio of the number of random hubs or bottlenecks proportions that are larger than Y to 10 000.

3 RESULTS

3.1 Overlapping target proteins and protein pairs between viral proteins and human miRNAs

Viruses may benefit by directly interacting with human proteins targeted by human miRNA (Fig. 1a); for example, miR-17-5p and miR-20a can repress the transcriptional coactivator of Tat during HIV infection (Triboulet *et al.*, 2007). To explore the direct relationship between viral proteins and human miRNAs, the overlap between host targets recognized by viral proteins and human miRNA targets was examined. Approximately 30% of the host targets recognized by viruses represent human miRNAs ($P < 0.0001$, Fisher's exact test). Contingency tables are shown in Supplementary Tables S1 and S2. The overlap is further described in Figure 2a, including representation of a network based on the systematic associations identified between viral proteins and human miRNAs (Fig. 2b).

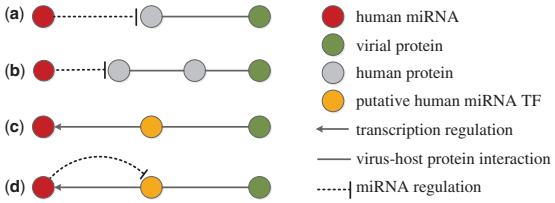

Fig. 1. Four typical patterns of human miRNA-mediated virus–host interactions. (**a**) A host target of a viral protein is also a target of a human miRNA. (**b**) A protein pair targeted by a human miRNA and a viral protein. (**c**) A viral protein targeting a host protein is a TF of a human miRNA. (**d**) A viral protein targeting a host protein (potentially a TF of a human miRNA) forming a feedback loop to a human miRNA

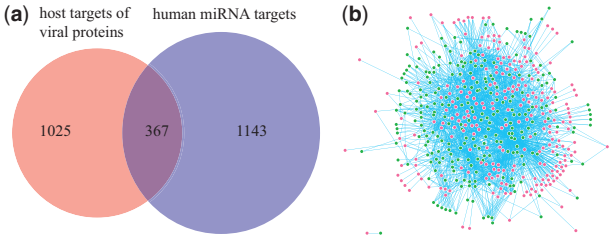

Fig. 2. Analysis of the targets shared by viral proteins and human miRNAs. (**a**) Venn diagram represention of the overlap of host targets recognized by virus and human miRNA targets. (**b**) An association network of human miRNAs (red circles) and viral proteins (green circles). A human miRNA was associated with a viral protein if its target interacted with viral proteins. The CYS formatted Cytoscape file of the human miRNA and viral protein association network and the interaction list are available in more detail in the Supplementary Files

It has previously been demonstrated that human miRNAs and viral proteins tend to interact with hubs and bottlenecks in human PPIN. To eliminate the influence of the degree and betweenness centrality of their overlap, we preserved the distribution of these variables, respectively, and re-examined their overlap. Results indicated that viruses may provide distinct contributions to the significant overlap (Supplementary Files).

Two interacted proteins can be targeted by a viral protein and a human miRNA, respectively (Fig. 1b), and therefore the miRNA may be involved into the virus infection process. For example, PI3 kinase and its activator ESR1 form an interacted protein pair. The PI3 kinase was revealed to be targeted by HCV NS5A protein, and can be activated during HCV infection (Street *et al.*, 2005). On the other hand, ESR1 was found to be a target of miR-18b (Yoshimoto *et al.*, 2011). It can then be speculated that overexpression of miRNA-18b may be unfavorable to HCV infection. In fact, miRNA-18b had been found to be downregulated during HCV infection process (Liu *et al.*, 2010), which may contribute to the overexpression of PI3 kinase. Herein, their relationships were investigated in the network view. On one hand, human protein interaction pairs targeted by viral proteins and human miRNAs were examined.

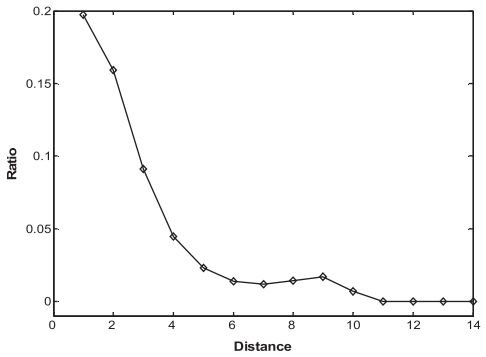

Fig. 3. The relationship between network distance and the ratio of protein pairs targeted by human miRNAs and viral proteins, respectively. These results demonstrate that the ratios decreased as the network distance increased ($P<0.0001$, Spearman rank correlation coefficient = −0.949)

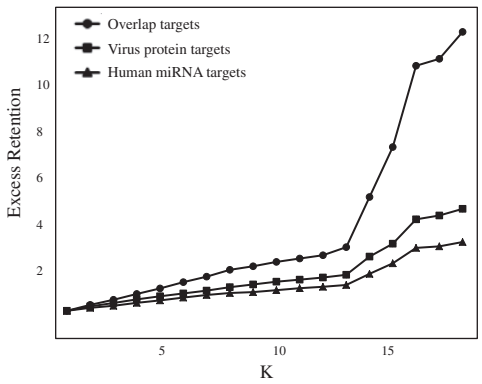

Fig. 4. A *k*-core analysis of overlapping targets, viral protein targets and human miRNA targets

A total of 7456 interaction pairs were found to be targeted by viral proteins and human miRNAs, whereas 7086 interaction pairs were targeted under random conditions. These results indicated that human PPI pairs represent significant targets of viral proteins and human miRNAs ($P<0.0001$). On the other hand, all human protein pairs were divided into 14 groups based on their network distances (the maximum distance of protein pairs was 14). It was observed that the ratio of protein pairs targeted by human miRNAs and viral proteins significantly decreased as the network distance increased (Fig. 3).

3.2 Topological features of the overlapping targets present in the PPIN

To further understand the relationship between viruses and human miRNAs, we analyzed the topological characteristics of the overlapping targets in the PPIN.

The proportion of hubs and bottlenecks in the overlapping targets was 32.43 and 29.70%, respectively, which are significantly higher percentages than observed under random conditions (Supplementary Tables S3 and S4). Compared with non-overlapping targets, the overlapping targets also had a greater degree and betweenness centrality ($P<0.0001$, Kolmogorov–Smirnov test). For example, the mean degrees of overlapping and non-overlapping targets were 30.8065 and 7.4968, respectively, whereas the mean betweenness centralities were 0.0020 and 0.0003, respectively. These results are consistent with previous findings, demonstrating that human miRNAs and viral proteins tend to interact with hubs and bottlenecks in the human PPIN (Dyer *et al.*, 2008; Hsu *et al.*, 2008).

Results from *k*-core and ER analyses further demonstrated that the ER value increased as *k* increased (Fig. 4) indicating that the overlapping targets were located in the global center of the human PPIN. The ER value of viral proteins and human miRNA targets showed similar trends, but had lower values at each step, indicating that the overlapping targets occupy deeper layers in the human PPIN. An additional function enrichment analysis (Thomas *et al.*, 2003) was performed indicating that the overlapping proteins were mainly involved in cellular processes such as cell communication and transport (Supplementary Fig. S1).

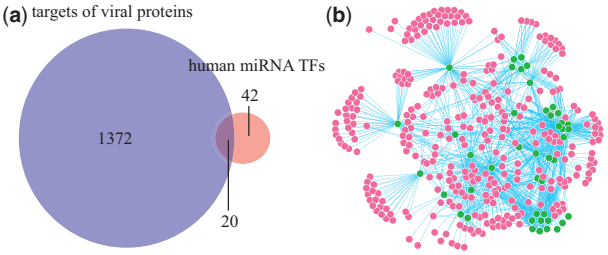

Fig. 5. Analysis of human miRNAs with regulators targeted by viral proteins. (**a**) Venn diagram representation of the overlap between host targets recognized by viruses and human miRNAs TFs. (**b**) An association network of human miRNA (red circles) and viral proteins (green circles) associated with a human miRNA if it targets miRNA TFs. The CYS formatted Cytoscape file of the human miRNA and viral protein association network and the interaction list are available in more detail in the supplementary files

3.3 Transcription factors of human miRNAs are preferentially targeted by viral proteins

Viral protein can regulate human TFs specific for human miRNAs (Fig 1c). For example, the LMP1 protein of Epstein–Barr virus can activate NF-κB, which was confirmed to be a transcription factor of miR-146a (Cameron *et al.*, 2008). For a better understanding of the relationships between viral proteins and human miRNAs, the significance of overlap between host targets recognized by viral proteins and human miRNA TFs was examined. This analysis demonstrated that 32.26% of human miRNA TFs were found to be targeted by viral proteins, and the overlap was significant based on the 2×2 contingency tables ($P=0.0006$; Fisher's exact test). The details are provided in Supplementary Table S3. Contingency tables are provided in Supplementary Tables S5 and S6. A Venn diagram of the overlap is illustrated in Figure 5a, and an association network of viral proteins and human miRNAs was constructed based on human miRNA regulators (Fig. 5b). Taken together, these results suggested that viruses potentially have the capacity to regulate expression of human miRNAs.

Negative transcriptional coregulation of an miRNA and its target is a recurring motif in mammals (Tsang *et al.*, 2007). For example, E2F and Myc, which are targets of miR-17-92,

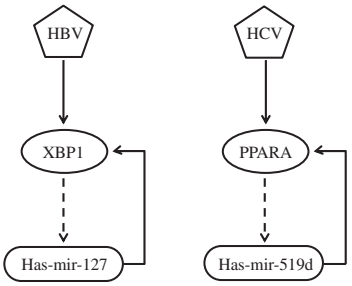

Fig. 6. Two potential feedback loops formed by the targeting of host proteins by viruses and human miRNAs

also induce miR-17-92 transcription (Aguda *et al*., 2008). Based on human miRNA TFs targeted by viral proteins, two potential feedback loops were identified that included host targets of viral proteins and human miRNAs (Fig. 1d). We hypothesized that these loops may be important in boosting the virus–host balance (Fig. 6).

The first potential feedback loop identified was related to hepatitis B virus (HBV) (Fig. 6, left). XBP1 is a protein that participates in the IRE1-XBP1 pathway involved in mediating the ER stress response or unfolded protein response. The HBV X protein can mediate activation of the unfolded protein response pathway that most likely promotes HBV replication and expression in liver cells (Li *et al*., 2007). Therefore, regulation of XBP1 by hsa-mir-127 may be used by HBV to fine–tune its expression. The second feedback loop identified was related to HCV (Fig. 6, right). It has been shown that PPARα activation plays a crucial role in HCV core protein-induced hepatic steatosis and hepatocellular carcinoma in mice (Tanaka *et al*., 2008). Therefore, PPARα inhibition by hsa-mir-519d may facilitate the regulation of HCV disease progression.

4 DISCUSSION

The discovery that human miRNAs play crucial roles in viral pathogenesis provides valuable insight into new patterns of virus–host interactions. Based on the systematic overlap analysis performed using data from virus–host protein interactions, previously identified human miRNA-target pairs and known human miRNA TFs, it appears that human miRNAs have important and wide ranging effects on virus–host interactions.

The existence of a large proportion of shared target proteins and protein pairs between viral proteins and human miRNAs indicates that human miRNA regulation subnetworks and viral infections are significantly interwoven in host cell networks. Correspondingly, analysis of topological characteristics associated with the PPIN reveals that overlapping proteins were preferentially topologically important (e.g. hubs and bottlenecks) and tended to occupy the global center of the PPIN. It is possible that the special position of the overlapping targets in the PPIN facilitates the ability of viruses and human miRNAs to efficiently take control of the PPIN. The functional analysis of overlapping targets also demonstrates that these genes take part in basic cellular processes, such as cell communication and apoptosis, which also suggests that human miRNAs play key roles in the process of virus infection and replication.

The significant overlap between host targets for viruses and possible human miRNA TFs also reveals a new pattern of virus–host interactions. Therefore, targeting human miRNA TFs may be an effective way for a virus to gain control of a host cell, especially because miRNAs can regulate a large number of host proteins. Interestingly, we also find that the host targets of viral proteins may form feedback loops with human miRNAs. These negative feedback loops may be exploited by viruses to fine–tune their pathogenesis and to complete their replication cycle. Therefore, interfering with these regulatory circuits may provide new approaches for developing antiviral therapies. Certainly, stronger experimental and computational evidence will be needed to further define the existence and universality of the described virus–host interaction patterns.

In this article, rules governing virus–host interactions were systematically analyzed using a high-quality dataset. Because of the complexity of virus–host interactions, all the possible virus–host interaction patterns could not be explored and only several typical virus–host interaction patterns involving the role of human miRNAs were described, and there are reports of the roles of host epigenetic regulators (Abraham and Kulesza, 2012; White *et al*., 2010). There are many other patterns involving virus–host interactions that need to be further investigated. For example, human miRNAs can directly target viral genomes for defense (Lecellier *et al*., 2005), and viral proteins were also found to regulate expression of human miRNAs that targeted host chromatin remodeling proteins (Rahman *et al*., 2012).

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