Research Overview

Infectomics Screening for Novel Antiviral Drug Targets

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ABSTRACT  Infectomics, a novel way to globally and comprehensively understand the interactions between microbial pathogens and their hosts, has significantly expanded understanding of the microbial infections. The infectomics view of viral–host interactions on the viral perspective principally focuses on gene acquisition, deletion, and point mutation, while traditional antiviral drug discovery concentrates on viral encoding proteins. Recently, high-throughput technologies, such as mass spectrometry-based proteomics, activity-based protein profiling, microarray analysis, yeast two-hybrid assay, small interfering RNA screening, and micro RNA profiling, have been gradually employed in the research of virus–host interactions. Besides, signaling pathways and cellular processes involved in viral–host interactions provide new insights of infectomics in antiviral drug discovery. In this review, we summarize related infectomics approaches in the studies of virus–host interactions, which shed light on the development of novel antiviral drug targets screening. Drug Dev Res 73 : 365–380, 2012.© 2012 Wiley Periodicals, Inc.

Key words: infectomics; drug targets; viral–host interactions

INTRODUCTION

Viruses, as small infectious agent, infect nearly all organisms and can cause severe infectious diseases in human. The use of chemical therapy (e.g., ribavirin) or vaccines has proved effective in protecting against viral infections, but some infections like human immunodeficiency virus (HIV) [Cohen et al., 2011], hepatitis B virus (HBV) [Dienstag, 2008], and influenza viruses [Lambert and Fauci, 2010] remain a great challenge. With progress in Genome Sequencing Project, the whole-genome complete genomes of 2,837 viruses have been sequenced at the time of writing this review (http://www.ncbi.nlm.nih.gov/genomes/GenomesGroup.cgi?taxid=10239&opt=Virus), leading to a new era of viral genomics and proteomics [Burley et al., 1999; Pandey and Mann, 2000; Dongre et al., 2001; Collins et al., 2003; Tyers and Mann, 2003]. Previous studies, mainly based on genomic and proteomic approaches, have made significant progress in establishing the foundation of network-based investigations on viral–host interactions [Chakravarti et al., 2000; Wilson and Richardson, 2005; Wu et al., 2005].

Infectomics, a term first introduced by Huang et al., 2002], is a novel means to globally and comprehensively understand interactions between microbial pathogens and their hosts rather than microbial pathogens themselves. Three types of infectomics approaches have been developed for antimicrobial drug discovery: ecological infectomics, immunoinfectomics, and chemical infectomics [Huang et al., 2007]. Among them, the accelerated development of chemical-based infectomics approaches has greatly contributed to drug discovery efforts. High-throughput approaches that

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include mass spectrometry (MS)-based proteomics, activity-based protein profiling (ABPP), microarray analysis, yeast two-hybrid (YTH) assays, small interfering RNA (siRNA) screening, and micro RNA (miRNA) profiling, are used to study the infections related to viral infection, leading to detailed mechanisms of viral–host interactions, including HIV [Brass et al., 2008; König et al., 2008; Naji et al., 2012], hepatitis C virus (HCV) [Supekova et al., 2008; Peng et al., 2009], influenza virus [König et al., 2009], and dengue virus (DENV) [Khadka et al., 2011].

Recent progress in antiviral drug research based on infectomics approaches has greatly revolutionized existing strategies. New antiviral compounds targeting host gene-encoding proteins or enzymes are in preclinical research, e.g., PRO2000 [McCormack et al., 2010] and bisindolylmaleimide [Ludwig et al., 2003]. As nuclear receptors play a combinatorial role in inflammation and immunity [Glass and Ogawa, 2005], novel drugs targeting these represent a potential strategy for antiviral therapy. Targeting infectome cellular proteins or molecules may lead to reduced host drug resistance, as the human genes encoding targeted cellular proteins are less likely to mutate in response to therapy [Tan et al., 2007]. Infectomics approaches can be used to study complex viral–host interactions to facilitate screening novel antiviral drug targets. In this review, we focus on high-throughput infectomic approaches in the study of virus–host interactions and their potential in antiviral drug discovery.

THE VIRAL PERSPECTIVE IN DRUG DISCOVERY

In the past few decades, many publications involving viral infections have focused on genomic and proteomic approaches from a viral perspective. The gene acquisition, deletion, and point mutation are three major events leading to the evolution of microbial pathogens or commensals. Point mutation is the most frequent leading to the promotion of viral replication and drug resistance. The survival ability of some viruses is enhanced due to viral point mutation, indicating that point mutations may play a critical role in virus spread. For example, in spite of the presence of antibodies against e antigen in the serum, patients infected with HBV still have a high titer of HBV DNA. Akahane et al. [1990] by sequencing the precore region of HBV, identified a point mutation at nucleotide 83 that was present in 98% clones of HBV propagated from the sera of seven patients, and another mutation at nucleotide 86 in 29 clones from two patients, which were responsible for dysfunctional secretion of e antigen. This point mutation is not limited to HBV, but is a general viral phenomenon that has been identified in a variety of viruses, including HIV [Emiliani et al., 1996], HCV [Heilke and Peterson, 1997], DENV [Hanley et al., 2003], influenza virus [Melikyan et al., 2000], and herpes simplex virus (HSV) [Hwang et al., 1992].

Using genomic and proteomic approaches, significant progress in drug discovery based on viral encoding proteins has occurred. Gel electrophoresis and liquid chromatography-MS/MS technologies resulted in the discovery of many proteins that may represent potential drug targets and include viral enzymes like reverse transcriptase (RT), integrase, and protease, which are involved in virus binding, reverse transcription, integration, and budding. For instance, the use of HIV-1 protease inhibitors, like saquinavir, ritonavir, indinavir, and nelfinavir have improved the treatment of HIV-infected patients [Deeks et al., 1997; Eron, 2000]. However, many patients treated with HIV-1 protease inhibitors developed tolerance [Condra et al., 1995; Yerly et al., 1999] requiring an enhanced knowledge of the landscape of viral infections. Furthermore, host factors that play essential roles in viral infections could also serve as novel antiviral drugs.

THE HOST PERSPECTIVE IN DRUG DISCOVERY

To date, virus–host interactions require additional characterization, as previous studies of viral infections focused mainly on the viruses themselves leading to antiviral drugs that targeted viral proteins with inherent limitations, e.g., rapid resistance due to the viral type and low fidelity of viral replication, especially for the RNA viruses [Drake et al., 1998] [Friedel and Haas, 2011]. Furthermore, viral genomes represent a limited number of drug targets [Tisoncik et al., 2009]. With novel high-throughput technologies, host factors essential for viral infections have been identified. A summary of the currently used high-throughput technologies and their contributions to antiviral drug discovery follows.

MS-Based Proteomics

Proteome analysis (primary sequence, protein–protein interactions, posttranslational modifications, etc.) can be used to study cellular states and determine molecular aspects of cellular function. Owing to the complexity of proteins and their low abundance, MS-based proteomics [Rabilloud, 2002; Montoliva and Albar, 2004; Righetti et al., 2004] is an indispensable tool in viral systems biology (Fig. 1A) [Aebersold and Mann, 2003]. Ion traps [Fenn et al., 1989; Pitteri et al., 2005; Makarov et al., 2006; Second et al., 2009; Shaner et al., 2009], time-of-flight (TOF) [Marklein et al., 2009; Seng et al., 2009; Prod’hom et al., 2010],
quadruple [Abzalimov and Kaltashov, 2010; Ramanathan et al., 2011], and Fourier transform-MS ion cyclotron analyzers [Leon et al., 2009; Allwood et al., 2012] are the four basic types of mass analyzers used in proteomic investigation. There are multiple options available to collect data and analysis including isotope-coded affinity tag [Haqqani et al., 2005], stable isotope labeling with amino acids in cell culture [Asara

| Organism | Proteome | Subproteome | 2DE | Protein species | Digestion | Mixture of peptides | MS | MS/MS | Database analysis |
|----------|----------|-------------|-----|----------------|-----------|-------------------|----|------|------------------|

**Fig. 1.** High-throughput technologies and omics approaches currently used in novel drug target screening. Six technologies are commonly used in drug discovery. (A) MS-based proteomic approach. (B) Schematic view of activity-based protein profiling (ABPP). Cell lysates are mixed with activity-directed chemical probes targeting candidate proteins, and then the tags of the probes could be detected by in-gel analysis. (C) Schematic view of protein microarray analysis. First, the artificial antigens are attached on the solid substrate. Then, the primary antibodies are deposited to the hapten. Finally, the primary antibodies bind to the secondary antibodies with the labels for detection. (D) Schematic view of yeast two-hybrid assay (YTH). The protein of interest (bait) is fused to a DNA-binding domain and transfected in a yeast host cell that contains a reporter gene controlled by this DNA-binding domain. The functional transcription factor (TF) will reconstitute upon the physical interaction between bait and prey proteins, leading to the activation of the reporter gene. (E) Schematic view of siRNA screening. Step I, preparation of siRNAs; step II, rearray siRNAs into 384-well plates for high-throughput screening; step III, transfection of siRNAs into target cell lines; and step IV assay phenotype by identifying the plate position (see black cell). (F) Schematic view of miRNA profiling. The total RNAs are hybridized with the miRNAs. Then the RNAs are labeled with the pCp-Cy3 for detection. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]
et al., 2008], and N15 [Osserman et al., 1957]. As more viral and host genomes are sequenced, MS-based proteomics is employed in research on virus–host interactions. Two-dimensional electrophoresis (2DE)/MS-based proteomics are powerful tools in infectomics research. Matrix-assisted laser desorption/ionization-TOF-MS has been successfully used in combination with 2DE for infectomics analyses of the Chlamydia pneumoniae elementary body in Hep-2 cells and C. trachomatis reticulate body in HeLa 229 cells [Huang et al., 2002]. Of the HCV nonstructural proteins, nonstructural protein 5A (NS5A) plays a critical role in RNA binding [Huang et al., 2005] and replication [Tarao et al., 2005; Targett-Adams et al., 2008], but its exact role remains unknown, particularly in virus–host interactions. Choi et al., 2004 identified heat shock protein 27 (HSP27) as a protein that specifically co-immnoprecipitated with NS5A but not with NS5B using MS analysis and other approaches. Moreover, the N-terminal regions of NS5A (aa 1–181) was found to interact with the 1–122 amino acid domain of HSP27. When heat shocked, HSP27 and NS5A co-localize to the endoplasmic reticulum suggesting an important role during heat shock. Kou et al., 2006 examined the possibility of host factors, that inhibit translation in cultured cells, to interact with NS4A [Labbus et al., 2002; Stasko et al., 2002]. Glutathione S-transferase-NS4A interacting protein was found to be congruent with human translation eukaryotic elongation factor 1A (eEF1A). Furthermore, the central domain from residues 21–34 of NS4A interacted with eEF1A, causing inhibition of both cap-dependent and HCV internal ribosomal entry site-mediated translation activities.

In HIV-1 infectious disease, MS-based proteomic research has also made significant progress. HIV-1 trans-activating (Tat) protein is necessary for viral replication and may play a critical part in HIV-1-associated diseases [Rasheed et al., 2009]. Interestingly, after HIV-1 infection, T cells were protected against apoptosis and survive longer bearing virions. Although the precise mechanism is unclear, it appears likely that HIV-1 Tat protein is responsible for such protection [Coiras et al., 2006]. Using MS–based proteomics, a number of cytoskeletal proteins, e.g., β-tubulin, actin, gelsolin, coflin, annexin II, and Rac/Rho-GDI (Rac and Rho-guanine nucleotide dissociation inhibitor) complex, were found to be downregulated by Tat protein. Reduced expression of these proteins limited the cytoskeletal changes induced by apoptosis and thus maintained HIV-1 virions. MS-based approaches have been used to screen potential inhibitors of Tat protein as novel therapeutic agents. Using nuclear magnetic resonance and MS/MS studies, [Jayasuriya et al., 2002 discovered durhamycin A as an inhibitor of Tat trans-activation. MS-based proteomics have explored other common viruses, including dengue [Pattanakitsakul et al., 2007; Higa et al., 2008], influenza [Williams et al., 2008; Schwahn et al., 2010], severe acute respiratory syndrome-associated coronavirus [Zeng et al., 2004; Jiang et al., 2005], and human respiratory syncytial virus [Brazier et al., 2004; Munday et al., 2010].

ABPP

ABPP technologies can monitor proteins or enzymes in their native environment, thus eliminating the need for recombinant expression, purification, and the development of a specific assay. Activity-directed chemical probes, which target many members of a given enzyme class or protein family, can be used to evaluate the activity of candidate compounds directly in complex proteomes. Identifying protein function and validating its biologic role is a prerequisite to lead discovery (Fig. 1B). The discovery of reversible enzyme inhibitors can be simplified with this chemical proteomic approach. In order to characterize the specific cellular functions of the cysteine proteases required for survival of the malaria parasite Plasmodium falciparum, a chemical proteomic screen was used to characterize these predominant proteases. Falcipain 1 was identified as the only active protease during the invasive merozoite stage. Specific inhibitors for falcipain 1 were then identified by the screening and were able to block parasite invasion of host erythrocytes, suggesting that falcipain 1 played a specific role in host cell invasion with the inhibitor for this enzyme representing a potential new agent for antimalarial therapeutics [Greenbaum et al., 2002].

Microarray Analysis

Microarray analysis, a 2D array on a solid substrate using high-throughput screening methods to assay large amounts of biological material [Barbulovic-Nad et al., 2006], was first used to study the small mustard plant Arabidopsis thaliana [Schena et al., 1995] and was then used to study yeast [Shalon et al., 1996], human [Schena et al., 1996], and mouse [Lockhart et al., 1996]. Compared with traditional approaches, the principal advantage of microarrays is that a large number of targets can be analyzed in parallel measurements with low sample consumption (Fig. 1C). Similar to recombinant DNA [Jackson et al., 1972] and polymerase chain reaction (PCR) [Mullis and Faloona, 1987], microarray is a seminal technology with broad application [Stears et al., 2003], including genomics [DeRisi et al., 1997; Hughes et al., 2000; Sudarsanam et al., 2000] and proteomics [Geiss et al., 2000; MacBeath and Schreiber, 2000; Eichhoff et al., 2002]. The two commonly used microarrays are those for DNA and protein [Templin et al., 2002].
where enzyme-substrate [Bulyk et al., 1999; Arenkov et al., 2000; MacBeath and Schreiber, 2000; Zhu et al., 2000], DNA-protein [Bulyk et al., 1999], protein-ligand, and different types of protein–protein interactions [Ge, 2000] are studied. DNA microarrays shifted direct antiviral screening programs to rational and genome-wide target-based strategies [Schmid, 2001; Chan et al., 2002; Fritz and Raczniaik, 2002; McDavitt et al., 2002; Parkinson, 2002; Cheng et al., 2003]. Since the first report of microarray-based investigations of HIV-induced alterations in host gene expression [Geiss et al., 2000], microarrays have been used in HIV studies [Izmailova et al., 2003; Khodakov et al., 2008] to investigate macrophage responses of infection by African swine fever virus [Zhang et al., 2006]. Genomic comparison of tuberculosis vaccine strain variants (Bacillus Calmette-Guérin), Mycobacterium tuberculosis H37Rv, Helicobacter pylori, and methicillin-resistant Staphylococcus aureus has been conducted using DNA microarray analysis providing new information on the evolution of these human pathogens suggesting rational approaches to the design of improved diagnostics and antimicrobial agents. However, bridging the gap between genomes and therapeutics is a challenging and time-consuming research process that is rate-limiting [Falb and Jindal, 2002; Zanders et al., 2002]. Protein microarrays also provide a high-throughput platform for target identification. A cluster of secreted proteins (α-defensins 1, 2, and 3) were identified as cell anti-HIV factors (CAFs) [Zhang et al., 2002] that were secreted by cluster of differentiation 8 T-lymphocytes from certain immunologically stable HIV-1 patients to suppress HIV-1 replication. The specific antibody recognition and amino acid sequencing were used to confirm the identity of CAF. Protein microarrays using malaria parasite surface proteins have been developed for studies of parasitic diseases [Bacarese-Hamilton et al., 2002]. The receptor-binding characteristics of two isolates of the novel pandemic H1N1 virus, Cal/09, and A/Hamburg/5/2009 (Ham/09), which were compared directly by carbohydrate microarray analysis [Childs et al., 2009].

**YTH Assay**

The YTH assay [Fields and Song, 1989] is a high-throughput technology to study protein–protein interactions [Fields, 2005]. In YTH, a protein of interest is fused to a DNA-binding domain and transfected in a yeast host cell with the reporter gene controlled by this DNA-binding domain. This fusion protein can be used as a “bait” or “target” to screen a library of cDNA clones fused to an activation domain. The functional transcription factor will reconstitute upon the physical interaction between bait and prey proteins, activating a reporter gene (Fig. 1D). YTH has been widely used in studying virus–host protein–protein interactions, including *Escherichia coli* bacteriophage 7 [Bartel et al., 1996], HIV [Rossi et al., 1996], and HCV [Matsunoto et al., 1997; Mamiya and Worman, 1999; Kittleisen et al., 2000]. Khadka et al. [2011] used a YTH assay to study network interactions between DENV and human proteins and validated a subset of these interactions through split-luciferase, siRNA, and colocalization experiments, resulting in the first genome-wide analysis of DENV–human protein–protein interactions. They identified 93 proteins required for DENV replication, 60 of which were novel and not been linked to any other viruses, and showed that some of these proteins were in DENV infection and also linked to other viruses, particularly HCV [Khadka et al., 2011]. This study has provided new light on DENV–host interactions as well as new potential drugs targeting host proteins. Mouse hepatitis virus (MHV)-68, a useful model for the study of human γ-herpes viruses, has been studied using YTH with 23 intraviral protein interactions and 243 virus-cellular protein interactions being identified, most of which have never been reported before [Lee et al., 2011]. Such studies may reveal potential cellular proteins that are utilized by MHV-68 or DENV, which may serve as new targets for therapeutic intervention. Studies on stomatitis virus [Moerdyk-Schauwecker et al., 2011], Sesbania mosaic virus [Chowdhury and Savithri, 2011], flavivirus [Le Breton et al., 2011], influenza virus [Sharma et al., 2011; Tafforeau et al., 2011], human cytomegalovirus [To et al., 2011], and human T-lymphotropic virus (HTLV) types 1 and 2 retroviruses [Simonis et al., 2012] also provided novel insights into virus–host interactions.

Variants of YTH include membrane YTH system, split-tobacco etch virus system, and mammalian protein–protein interaction trap [Suter et al., 2008]. Compared with YTH, new technologies like reverse YTH and the yeast three-hybrid system, can provide more integrative data to understand of viral–host interactions and provide new strategies for antiviral drug discovery. For instance, inhibitors of dimerization can be used to disrupt protein–protein interactions induced by viral infections, which may prevent viral infection or replication. Protease, RT, invertase of HIV, and DNA polymerase of HSV, which play critical roles in HIV and HSV infections, are drug targets for HIV and HSV therapies [Archakov et al., 2003].

**siRNA Screening**

RNA interference (RNAi) is a mechanism within living cells to modulate gene activity [Fire et al., 1998]. It is widely used to study gene function and associated
molecular mechanisms [Hannon, 2002; Mello and Conte, 2004]. Since initial studies on large-scale RNAi screening in Caenorhabditis elegans [Fraser et al., 2000; Gönczy et al., 2000], RNAi screening has been routinely used to study of pathogen–host interactions, enabling genome-scale loss of function screening in host cells (Fig 1E) [Echeverri and Perrimon, 2006; Boutros and Ahringer, 2008; Mohr et al., 2010; Ou et al., 2012]. Genome-wide RNAi screening identified 287 human host cell genes influencing influenza A virus replication [Karlas et al., 2010] with host protein p27, a cell cycle regulator, being identified as key for influenza virus replication. A small molecule inhibitor of cell division cycle-like kinase 1 reduced influenza virus replication. Another study identified 295 cellular cofactors required for early-stage influenza virus replication with 23 factors necessary for viral entry confirmed [König et al., 2010]. Some 250 host cellular factors influencing HIV-1 infection have been identified 40 factors participating in the early stage of HIV infection [Brass et al., 2008; König et al., 2008]. High-throughput RNAi screening has identified host factors required in virus progression, including those for HCV [Li et al., 2009; Tai et al., 2009], West Nile virus [Krishnan et al., 2008], DENV [Sessions et al., 2009], and drosophila C virus [Cherry et al., 2005].

**miRNA Profiling**

miRNAs [Lee et al., 1993] play important roles in the control of stress signaling [Mendell and Olson, 2012], metabolism [Rottiers and Naar, 2012], tumorigenesis [Chen, 2005], and viral–host interactions [Jopling et al., 2005; Lecellier et al., 2005]. High-throughput miRNA profiling technologies, including quantitative reverse transcription PCR-based methods, hybridization-based methods, RNA-seq, and pri- and pre-miRNA quantification [Pritchard et al., 2012] have greatly enhanced knowledge regarding the role of miRNA in viral–host interactions (Fig 1F). miRNA profiling technologies combined with messenger RNA (mRNA) profiling have provided new insight into HCV–host interactions. Investigation of miRNAs and mRNAs involved in HCV infection identified 43 differentially expressed miRNAs and 6,850 differentially expressed mRNAs expression levels of which were changed during HCV infection [Liu et al., 2010; Steuerwald et al., 2010]. These altered expression levels of miRNAs and mRNAs were involved in metabolism, cell growth, apoptosis, and cytokine/chemokine pathways, and in the progression of HCV-induced chronic hepatitis. Another study revealed 10 miRNAs that were downregulated in Hep-394 cells with 23 miRNAs upregulated [Braconi et al., 2010]. The identified miRNAs and their putative targets may be used as the basis for anti-HCV therapies, suggesting that combined miRNA and mRNA profiling may represent a novel approach to understand HCV infection and design new anti-HCV strategies.

Antisense inhibitors of miRNA function, that are bioavailable in vivo, e.g., antagomirs, represent a good starting point for the development of miRNA inhibitory drugs [Krutzfeldt et al., 2005; Gottwein et al., 2007]. However, antisense inhibitors targeting cellular miRNAs have been predicted to have side effects, as these inhibitors could also disrupt cellular functions of these miRNAs. Moreover, like treatment with other antiviral drugs, the virus may become resistant, e.g., through mutation of the viral miRNAs or viral binding sites for cellular miRNAs [Gottwein and Cullen, 2008]. In this case, miRNA-based therapeutics would be needed to be used in combination with other antiviral drugs.

**NEW INSIGHTS OF INFECTOMICS IN ANTIVIRAL DRUG DISCOVERY**

Because of their relatively small genome, viruses must interact with host-encoding proteins to “hijack” host cellular signaling pathways and cellular processes, e.g., apoptosis, autophagy, and metabolism, to evade the host defense system and create a suitable microenvironment for their rapid replication [Bowie et al., 2004; Iannello et al., 2006; Galluzzi et al., 2010; Kaminsky and Zhitovtovskyy, 2010]. Many groups have thus focused on host perspective to identify host factors involved in viral infection using system or network biology approaches have been used in studying viral infection and many cellular signaling pathways [de Chassy et al., 2008; Pauli et al., 2008; Shapira et al., 2009; Jia et al., 2010].

**Signaling Pathways Involved in Viral–Host Interactions**

Using high-throughput technologies (Table 1), new insights into virus–host interactions have occurred that suggest that host cellular signaling pathways may be hijacked or promoted by virus infection, making viral–host interactions more complex than previously thought. Receptors on the cell membrane, adaptor molecules in the cytoplasm, and nuclear transcription factors [Dai et al., 2011] represent three categories of host signaling molecules that play important roles in virus–host interactions [DeLarco and Todaro, 1976; Albritton et al., 1989; Doria et al., 1995; Choe et al., 1996; Yoneyama et al., 1998; Waris et al., 2001; Hemmi et al., 2004; Perry et al., 2004] (Fig 2).
| Approaches                             | Characteristics                                                                 | Viruses been studied            | References                                      |
|----------------------------------------|---------------------------------------------------------------------------------|---------------------------------|-------------------------------------------------|
| MS-based proteomics                    | 1. Investigate high degree complexity of protein                                | HCV                             | [Choi et al., 2004; Kou et al., 2006]           |
|                                        | 2. Research low abundance of some proteins                                       | HIV-1                           | [Jayasuriya et al., 2002]                      |
|                                        | 3. Identify the observed proteins                                               | Dengue virus                    | [Pattanakitsakul et al., 2007; Higa et al., 2008] |
|                                        |                                                                                 | Influenza virus                 | [Williams et al., 2008; Schwahn et al., 2010]  |
|                                        |                                                                                 | SARS-CoV                        | [Zeng et al., 2004; Jiang et al., 2005]        |
|                                        |                                                                                 | RSV                             | [Braisier et al., 2004; Munday et al., 2010]   |
| Activity-based protein profiling       | 1. Determine the changes in the catalytic state of enzymes in complex proteomes | HCV                             | [Singaravelu et al., 2010; Blais et al., 2010a,b] |
|                                        | 2. Ascribe previously unknown enzymatic functions to proteins                   | HPV-1                           | [Rolen et al., 2006]                           |
|                                        | 3. Effective in targeting enzyme families with known covalent inhibitors         | SARS-CoV                        | [Shah et al., 2010]                            |
|                                        |                                                                                 | HSV-1                           | [Kattenhorn et al., 2005]                      |
|                                        |                                                                                 | H1N1 virus                      | [Marmett et al., 2004]                         |
| Microarray analysis                    | 1. Multiplex lab-on-a-chip                                                       | HBV and HCV                     | [Iizuka et al., 2002]                          |
|                                        | 2. 2D array on a solid substrate                                                | H1N1 virus                      | [Childs et al., 2009]                          |
|                                        | 3. Genetic analysis, mRNA level analysis, and protein level analysis             | HIV                             | [Geiss et al., 2000; Izmailova et al., 2003; Khodakov et al., 2008] |
|                                        | 4. A large number of targets can be analyzed in massive parallel measurements   | ASFV                            | [Zhang et al., 2006]                           |
|                                        | 5. Low sample consumption                                                       | Parvovirus B19                  | [Kerr, 2005]                                   |
|                                        |                                                                                 | Human influenza A and B viruses  | [Li et al., 2001]                               |
| Yeast two-hybrid assays                | 1. Low-tech, without sophisticated equipment                                     | HCMV                            | [To et al., 2011]                              |
|                                        | 2. Widely used in study protein–protein interaction                             | HCV                             | [Kittlesen et al., 2000]                       |
|                                        | 3. Scalable and time saving                                                     | Dengue virus                    | [Khadka et al., 2011]                          |
|                                        |                                                                                 | Stomatitis virus                | [Moerdyk-Schauwecker et al., 2011]             |
|                                        |                                                                                 | Influenza virus                 | [Sharma et al., 2011; Taforeau et al., 2011]   |
|                                        |                                                                                 | HTLV                            | [Simonis et al., 2012]                         |
| RNAi screening                         | 1. Rapid, unbiased, and large scale                                             | Influenza virus                 | [Karlas et al., 2010]                         |
|                                        | 2. Independent of any preconceived models                                        | HIV                             | [Brass et al., 2008; König et al., 2008]       |
|                                        | 3. Independent assumptions about gene functions                                  | West Nile virus                 | [Krishnan et al., 2008]                       |
|                                        |                                                                                 | Dengue virus                    | [Sessions et al., 2009]                       |
|                                        |                                                                                 | DCV                             | [Cherry et al., 2005]                          |
| miRNA profiling                        | 1. High-throughput technology                                                    | HCV                             | [Braconi et al., 2010; Steuerwald et al., 2010]|
|                                        | 2. Can be combined with miRNA profiling                                         | MHV-68                          | [Zhu et al., 2010]                             |
|                                        | 3. Expressive                                                                    | Adenovirus                      | [Qi et al., 2010]                              |
|                                        |                                                                                 | Enterovirus                     | [Cui et al., 2010]                             |

HHV, human herpes virus.
Overview of host signaling and cellular processes involved in viral infection. Integrated signaling networks functionally regulate the cellular processes of viral infection; implicated signaling pathways include NF-κB pathway, apoptotic pathway, autophagic pathway, and glucose metabolic pathway. Lines with an arrowhead indicate functional activation. Lines with a blunt end indicate functional inhibition. Ad12, human adenovirus 12; ATP, adenosine-5'-triphosphate; BFV, bovine foamy virus; CAPS, capase; CD4, cluster of differentiation 4; CXCR4, chemokine (C-X-C motif) receptor 4; EHV, equine herpes virus; EMP, Embden–Meyerhofer–Pamas pathway; EV, ectromelia virus; FADD, Fas-associated protein with death domain; HCMV, human cytomegalovirus; HPV, human papillomavirus; HRSV, human respiratory syncytial virus; ICP34.5, glycoprotein A of varicella-zoster virus. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

miRNAs could participate in the regulation of host signaling pathways. This suggests that not only the viral proteins but also miRNAs could participate in the regulation of host signaling pathways.
strategies to target these processes for combating viral infections (Fig. 2).

CONCLUSION

With the many novel technology platforms becoming available, omics approaches have been widely used in drug discovery. Studies based on genomics and proteomics approaches have established the foundation of network-based investigations for viral-host interactions, creating opportunities for the development of novel antimicrobial agents. Six infectionomics technologies commonly used for drug discovery have been described in this review: MS-based proteomics, ABPP, microarray analysis, YTH assays, siRNA screening, and miRNA profiling. Together, they have the potential to elucidate and integrate the dynamic interactions between microbial pathogens and their hosts during the development of infectious diseases. Chemical infectionomics, which has the advantages of both high-throughput chemistry and infectionomics, is an emerging method for validating drug targets and will revolutionize approaches to infectious diseases. The infectionomics view provides: (i) a global detection and integrative dissection of microbial and host infections, that is critical to understanding the process of microbial pathogenesis and developing better diagnostic or therapeutic approaches for infectious diseases; (ii) a powerful tool to investigate microbial and human genomes to address the present crisis in antibiotic resistance; (iii) a new method of utilizing pharmacomes (lipid-based drug delivery systems) for optimal drug therapy; and (iv) the opportunity to exploit probiotics as ecological approaches to infectious diseases. With such tools, prevention and treatment of microbial infections will eventually enter an era when holistic solutions to health problems can be efficiently individualized.

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REFERENCES

Abzalimov RR, Kaltashov IA. 2010. Controlling hydrogen scrambling in multiply charged protein ions during collisional activation: implications for top-down hydrogen/deuterium exchange MS utilizing collisional activation in the gas phase. Anal Chem 82:942–950.

Aebbersold R, Mann M. 2003. Mass spectrometry-based proteomics. Nature 422:198–207.

Ait-Goughoulte M, Kanda T, Meyer K, Ryerse JS, Ray RB, Ray R. 2008. Hepatitis C virus genotype 1a growth and induction of autophagy. J Virol 82:2241–2249.

Akahane Y, Yamanaka T, Suzuki H, Sugai Y, Tasuda F, Yotsumoto S, Oni S, Okamoto H, Miyakawa Y, Mayumi M. 1990. Chronic active hepatitis with hepatitis B virus DNA and antibody against e antigen in the serum. Disturbed synthesis and secretion of e antigen from hepatocytes due to a point mutation in the precore region. Gastroenterology 99:1113–1119.

Alavian S, Ande S, Coombs K, Yeganeh B, Davoodpour P, Hashemi M, Los M, Ghavami S. 2011. Virus-triggered autophagy in viral hepatitis-possible novel strategies for drug development. J Viral Hepat 18:821–830.

Allbritton LM, Tseng L, Scadden D, Cunningham JM. 1989. A putative murine ecotropic retrovirus receptor gene encodes a multiple membrane-spanning protein and confers susceptibility to virus infection. Cell 57:659–666.

Allwood JW, Parker D, Beckmann M, Draper J, Goodacre R. 2012. Fourier transform ion cyclotron resonance mass spectrometry for plant metabolite profiling and metabolite identification. Methods Mol Biol 860:157–176.

Archakov AI, Govorun VM, Dubanov AV, Ivanov YD, Veselovsky AV, Levi P, Janssen P. 2003. Protein-protein interactions as a target for drugs in proteomics. Proteomics 3:380–391.

Arenkov P, Kukhtin A, Gemmell A, Voloshchuk S, Chupeeva V, Mirzabekov A. 2000. Protein microchips: use for immunoassay and enzymatic reactions. Anal Biochem 278:123–131.

Asara JM, Christofk HR, Freimark LM, Cantley LC. 2008. A label-free quantification method by MS/MS TIC compared to SILAC and spectral counting in a proteomics screen. Proteomics 8:994–999.

Bacarese-Hamilton T, Bistoni F, Crisanti A. 2002. Protein microarrays: from serodiagnosis to whole proteome scale analysis of the immune response against pathogenic microorganisms. Biotechniques (Suppl):24–29.

Barbulovic-Nad I, Lucente M, Sun Y, Zhang M, Wheeler AR, Bussmann M. 2006. Bio-microarray fabrication techniques—a review. Crit Rev Biotechnol 26:237–259.

Bartel PL, Roecklein JA, SenGupta D, Fields S. 1996. A protein linkage map of Escherichia coli bacteriophage T7. Nat Genet 12:75–77.

Bartholomeusz C, Gonzalez-Angulo AM. 2012. Targeting the PI3K signaling pathway in cancer therapy. Expert Opin Ther Targets 16:121–130.

Battaglia S, Benzoubir N, Noblet S, Charneau P, Samuel D, Zigueno AL, Atfi A, Brechet C, Bourgeade MF. 2009. Liver cancer-derived hepatitis C virus core proteins shift TGF-beta responses from tumor suppression to epithelial-mesenchymal transition. PLoS ONE 4:e4355.

Blais DR, Brulotte M, Qian Y, Belanger S, Yao SQ, Pezacki JP. 2010a. Activity-based proteome profiling of hepatoma cells during hepatitis C virus replication using protease substrate probes. J Proteome Res 9:912–923.

Blais DR, Lyn RK, Joyce MA, Rouleau Y, Steenbergen R, Barsby N, Zhu LF, Pegoraro AF, Stolow A, Tyrrell DL, et al. 2010b. Activity-based protein profiling identifies a host enzyme, carboxylesterase 1, which is differentially active during hepatitis C virus replication. J Biol Chem 285:25602–25612.

Boutros M, Ahiringer R. 2008. The art and design of genetic screens: RNA interference. Nat Rev Genet 9:554–566.

Bowie AG, Zhan J, Marshall WL. 2004. Viral appropriation of apoptotic and NF-kappaB signaling pathways. J Cell Biochem 91:1099–1108.
Chowdhury SR, Savithri HS. 2011. Interaction of Sesbania mosaic virus proteins modulate microRNA expression and chemosensitivity in malignant hepatocytes. Clin Cancer Res 16:957–966.

Brasier AR, Spratt B, Wu Z, Boldogh I, Zhang Y, Garofalo RP, Casola A, Pashmi J, Haag A, Luxon B, et al. 2004. Nuclear heat shock response and novel nuclear domain 10 reorganization in respiratory syncytial virus-infected A549 cells identified by high-resolution two-dimensional gel electrophoresis. J Virol 78:11461–11476.

Brass AL, Dykshoorn DM, Benita Y, Yan N, Engelman A, Xavier RJ, Lieberman J, Elledge SJ. 2008. Identification of host proteins required for HIV infection through a functional genomic screen. Science 319:921–926.

Bulyk ML, Gentelen E, Lockhart DJ, Church GM. 1999. Quantifying DNA–protein interactions by double-stranded DNA arrays. Nat Biotechnol 17:573–577.

Burley SK, Almo SC, Bonanno JB, Capel M, Chance MR, Gaasterland T, Lin D, Sali A, Studier FW, Swaminathan S. 1999. Structural genomics: beyond the human genome project. Nat Genet 23:151–158.

Ceconi F, Levine B. 2008. The role of autophagy in mammalian development: cell makeover rather than cell death. Dev Cell 15:344–357.

Chakravarti DN, Fiske MJ, Fletcher LD, Zagursky RJ. 2000. Application of genomics and proteomics for identification of bacterial gene products as potential vaccine candidates. Vaccine 19:601–612.

Chan PF, Macarron B, Payne DJ, Zalacain M, Holmes DJ. 2002. Characterization of hepatitis C virus NS5A protein. Biochem Biophys Res Commun 295:132–136.

Cheng Q, Wang S, Salyers AA. 2003. New approaches for antisense RNA-mediated gene silencing. Nat Biotechnol 21:555–561.

Cherry S, Doukas T, Armknecht S, Melffen G, Pradezynski F, Farla BF, Chantier T, et al. 2008. Hepatitis C virus infection protein network. Mol Syst Biol 4:230.

Chen CZ. 2005. MicroRNAs as oncogenes and tumor suppressors. N Engl J Med 353:1768–1771.

Cheng Q, Wang S, Salyers AA. 2003. New approaches for anti-infective drug discovery: antibiotics, vaccines and beyond. Curr Drug Targets Infect Disord 2:291–308.

de Chassey B, Navratil V, Tafforeau L, Hiet MS, Aublin-Gex A, Echeverri CJ, Perrimon N. 2006. High-throughput RNAi screening for new approaches for anti-HIV-1 Tat protein expression. Proteomics 6(Suppl 1):S63–S73.

Collins FS, Morgan M, Patrinos A. 2003. The Human Genome Project: lessons from large-scale biology. Science 300:286–290.

Dai X, Zhang L, Hong T. 2011. Host cellular signaling induced by influenza virus. Sci China Life Sci 54:68–74.

Deeks SG, Smith M, Holodniy M, Kahn JO. 1997. HIV-1 protease inhibitors. JAMA 277:145–153.

DeLarco J, Todaro GJ. 1976. Membrane receptors for murine leukemia viruses: characterization using the purified viral envelope glycoprotein, gp71. Cell 8:365–371.

DeRisi JL, Iyer VR, Brown PO. 1997. Exploring the metabolic and genetic control of gene expression on a genomic scale. Science 278:680–686.

Dienstag JL. 2008. Hepatitis B virus infection. N Engl J Med 359:1486–1500.

ten Dijke P, Hill CS. 2004. New insights into TGF-beta-Smad signalling. Trends Biochem Sci 29:265–273.

Dongre AR, Klein N, Lucito R, Schneider RJ. 2001. Proteomics in the post-genome age. Biopolymers 60:206–211.

Doria M, Klein N, Lucito R, Schneider RJ. 1995. The hepatitis B virus HBx protein is a dual specificity cytoplasmic activator of Ras and nuclear activator of transcription factors. EMBO J 14:4747–4757.

Draey JR, Charlesworth B, Charlesworth D, Crow JF. 1998. Rates of spontaneous mutation. Genetics 148:1667–1686.

Echeverri CJ, Perrimon N. 2006. High-throughput RNAi screening in cultured cells: a user's guide. Nat Rev Genet 7:373–384.

Eickhoff H, Konthur Z, Lueking A, Lehrach H, Walter G, Nordhoff N. 2002. Protein array technology: the tool for identification of potential targets of novel anti-viral drugs. J Mol Med 80:155–165.

Eichhorn H, Kontur Z, Leucking A, Lehrach H, Walter G, Nordhoff N, Nyarsik L, Bussow K. 2002. Protein arrays technology: the tool to bridge genomics and proteomics. Adv Biochem Eng Biotechnol 77:103–112.

Emilian S, Van Lint C, Fischle W, Paras P Jr, Ott M, Brady J, Verdin E. 1996. A point mutation in the HIV-1 Tat responsive element is associated with postintegration latency. Proc Natl Acad Sci U S A 93:6377–6381.

Eron JJ Jr. 2000. HIV-1 protease inhibitors. Clin Infect Dis 30(Suppl 2):S160–S170.

Falb D, Jindal S. 2002. Chemical genomics: bridging the gap between the proteome and therapeutics. Curr Opin Drug Discov Devel 5:323–339.

Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. 1989. Electrospray ionization for mass spectrometry of large biomolecules. Science 246:64–71.

Fields S. 2005. High-throughput two-hybrid analysis. The promise and the peril. FEBS J 272:5391–5399.
Hemmi H, Takeuchi O, Sato S, Yamamoto M, Kaisho T, Sanjo H, Heike GM, Peterson MG. 1997. A point mutation abolishes the
Hannon GJ. 2002. RNA interference. Nature 418:244–251.
Hanley KA, Mannucchi LA, Gilmore LE, Blaney JE Jr, Hanson CT, Greenbaum DC, Baruch A, Grainger MR, Bozdech Z, Medzihradszky
Gottwein E, Gottwein E, Cullen BR. 2008. Viral and cellular microRNAs as determinants of viral pathogenesis and immunity. Cell Host Microbe 3:375–387.
Gottwein E, Kellen C, Schlecht C, Bluemlein WH, Chi JT, Braesch T, Manoharan M, Soutschek J, Olden U, et al. 2007. A viral microRNA functions as an orthologue of cellular miR-155. Nature 450:1096–1099.
Greenbaum DC, Baruch A, Grainger MR, Bozdech Z, Medzihradszky KA, Engel J, DeRisi J, Holder AA, Bogyo M. 2002. A role for the protease falcipain 1 in host cell invasion by the human malaria parasite. Science 298:2002–2006.
Hanley KA, Manlucu LR, Gilmore LE, Blaney JE Jr, Hanson CT, Murphy BR, Whitehead SS. 2003. A trade-off in replication in mosquito versus mammalian systems conferred by a point mutation in the NS4B protein of dengue virus type 4. Virology 312:222–232.
Hannon GJ. 2002. RNA interference. Nature 418:244–251.
Hampi AS, Nesic M, Preston E, Baumann E, Kelly J, Stanimirovic D. 2005. Characterization of vascular protein expression patterns in cerebral ischemia/reperfusion using laser capture microdissection and ICAT-nanoLC-MS/MS. FASEB J 19:1809–1821.
Heilek GM, Peterson MG. 1997. A point mutation abolishes the helicase but not the nucleotide triphosphatase activity of hepatitis C virus NS3 protein. J Virol 71:6264–6266.
Hemmi H, Takeuchi O, Sato S, Yamamoto M, Kaisho T, Sanjo H, Kawai T, Hoshino K, Takeda K, Akira S. 2004. The roles of two
IkappaB kinase-related kinases in lipopolysaccharide and double stranded RNA signaling and viral infection. J Exp Med 199:1641–1650.
Higa LM, Caruso MB, Canellas F, Soares MR, Oliveira-Carvalho AL, Chapeaurouge DA, Almeida PM, Perales J, Zingali RB, Da Poian AT. 2008. Secretome of HepG2 cells infected with dengue virus: implications for pathogenesis. Biochim Biophys Acta 1784:1607–1616.
Huang L, Jiang X, Sharma SD, Hargittai MR, Chen Y, Arnold JJ, Raney KD, Cameron CE. 2005. Hepatitis C virus nonstructural protein 5A (NS5A) is an RNA-binding protein. J Biol Chem 280:36417–36425.
Huang SH, Triche T, Jong AJ. 2002. Infectomics: genomics and proteomics of microbial infections. Funct Integr Genomics 1:331–344.
Huang SH, Wang X, Jong AJ. 2007. The evolving role of infectomics in drug discovery. Expert Opin Drug Discov 2:961–975.
Hughes TR, Roberts CJ, Dai H, Jones AR, Meyer MR, Slade D, Burchard J, Dow S, Ward TH, Kidd MJ, et al. 2000. Widespread aneuploidy revealed by DNA microarray expression profiling. Nat Genet 25:333–337.
Hugle T, Fehrmann F, Bieck E, Kohara M, Krausslich HG, Rice CM, Blum HE, Moradpour D. 2001. The hepatitis C virus nonstructural protein 4B is an integral endoplasmic reticulum membrane protein. Virology 284:70–81.
Hwang CB, Ruffner KL, Coen DM. 1992. A point mutation within a distinct conserved region of the herpes simplex virus DNA polymerase gene confers drug resistance. J Virol 66:1774–1776.
Iannelli A, Delebeche O, Martin E, Attalah LH, Samaranis A, Ahmad A. 2006. Viral strategies for evading antiviral cellular immune responses of the host. J Leukoc Biol 79:16–35.
Izuka N, Oku M, Yamada-Okabe H, Mori N, Tamesa T, Okada T, Takemoto N, Takeda K, Nakayama H, et al. 2002. Comparison of gene expression profiles between hepatitis B virus- and hepatitis C virus-infected hepatocellular carcinoma by oligonucleotide microarray data on the basis of a supervised learning method. Cancer Res 62:3939–3944.
Izmailova E, Bertley FM, Huang Q, Makori N, Miller CJ, Young RA, Aldovini A. 2003. HIV-1 Tat reprograms immature dendritic cells to express chemotactants for activated T cells and macrophages. Nat Med 9:191–197.
Jackson DA, Symons RH, Berg P. 1972. Biochemical method for inserting new genetic information into DNA of simian virus 40: circular SV40 DNA molecules containing lambda phage genes and the galactose operon of Escherichia coli. Proc Natl Acad Sci USA 69:2904–2909.
Jayasuriya H, Lingham RB, Graham P, Quinnina D, Herranz L, Geuilloud O, Gagliardi M, Danzeisen R, Tomassini JE, Zink DL, et al. 2002. Durhamycin A, a potent inhibitor of HIV Tat transactivation. J Nat Prod 65:1091–1095.
Jia D, Bahbar R, Chan RW, Lee SM, Chan MC, Wang BX, Baker DP, Sun B, Peiris JS, Nicholls JM, et al. 2010. Influenza virus non-structural protein 1 (NS1) disrupts interferon signaling. PLoS ONE 5:e13927.
Jiang XS, Tang LY, Dai J, Zhou H, Li SJ, Xia QC, Wu JB, Zeng R. 2005. Quantitative analysis of severe acute respiratory syndrome (SARS)-associated coronavirus-infected cells using proteomic approaches: implications for cellular responses to virus infection. Mol Cell Proteomics 4:902–913.
Mamiya N, Worman HJ. 1999. Hepatitis C virus core protein binds to a DEAD box RNA helicase. J Biol Chem 274:15751–15756.

Marklein G, Josten M, Klanke U, Muller E, Horre R, Maier T, Wenzel T, Kostrzewa M, Bierbaum G, Hoerauf A, et al. 2009. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for fast and reliable identification of clinical yeast isolates. J Clin Microbiol 47:2912–2917.

Marnett AB, Nomura AM, Shimba N, Ortiz de Montellano PR, Craik AB, Nomura AM, Shimba N, Ortiz de Montellano PR, Craik. 2004. Revealing the world of RNA interference. Nature 431:338–342.

McCormack S, Ramjee G, Kamali A, Rees H, Crook AM, Gafos M, Jentsch U, Pool R, Chisembele M, Kapiga S, et al. 2010. PR02000 vaginal gel for prevention of HIV-1 infection (Microbicides Development Programme 301): a phase 3, randomised, double-blind, parallel-group trial. Lancet 376:1329–1337.

McDevitt D, Payne DJ, Holmes DJ, Rosenberg M. 2002. Novel targets for the future development of antibacterial agents. Symp Ser Soc Appl Microbiol 28S:348.

Melikyan GB, Markosyan RM, Roth MG, Cohen FS. 2000. A point mutation in the transmembrane domain of the hemagglutinin of influenza virus stabilizes a hemifusion intermediate that can transit to fusion. Mol Biol Cell 11:3765–3775.

Mello CC, Conte D. 2004. Revealing the world of RNA interference. Nature 431:338–342.

Mendell JT, Olson EN. 2012. MicroRNAs in stress signaling and human disease. Cell 148:1172–1187.

Moerdyk-Schanwecker M, Destephantis D, Hastie E, Grdzelishvili VZ. 2011. Detecting protein-protein interactions in vesicular stomatitis virus using a cytoplasmic yeast two hybrid system. J Virol Methods 173:203–212.

Mohr S, Bakal C, Perrimon N. 2010. Genomic screening with RNAi: results and challenges. Annu Rev Biochem 79:37–64.

Monteoliva L, Albar JP. 2004. Differential proteomics: an overview of gel and non-gel based approaches. Brief Funct Genomic Proteomic 3:220–239.

Mullis KB, Falooma FA. 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods Enzymol 155:335–350.

Munday DC, Emmott E, Surtees R, Lardeau CH, Wu W, Duprex WP, Dove BK, Barr JN, Hiscox JA. 2010. Quantitative proteomic analysis of A549 cells infected with human respiratory syncytial virus. Mol Cell Proteomics 9:2438–2459.

Murata M, Matsuzaki K, Yoshida K, Sekimoto G, Tahashi Y, Mori S, Uemura Y, Sakaida N, Fujisawa J, Seiki T, et al. 2009. Hepatitis B virus X protein shifts human hepatic transforming growth factor (TGF)-beta signaling from tumor suppression to oncogenesis in early chronic hepatitis B. Hepatology 49:1203–1217.

Naji S, Ambrus G, Cimermancic P, Reyes JR, Johnson JR, Fillbrandt R, Huber MD, Vesely P, Krogan NJ, Yates JR 3rd, et al. 2012. Host cell interaction of HIV-1 Rev includes RNA helicases involved in multiple facets of virus production. Mol Cell Proteomics 11:M111–015313.

Noch E, Sariyer IK, Gordon J, Khalli K. 2012. JC virus T-antigen regulates glucose metabolic pathways in brain tumor cells. PLoS ONE 7:e35054.

Orvedahl A, Levine B. 2009. Autophagy in Mammalian antiviral immunity. Curr Top Microbiol Immunol 335:267–285.

Orvedahl A, MacPherson S, Sumpter JR, Talloczy Z, Zou Z, Levine B. 2010. Autophagy protects against Sindbis virus infection of the central nervous system. Cell Host Microbe 7:115–127.

Osserman EF, Graff A, Marshall M, Lavaldr D, Graff S. 1957. Incorporation of N15-L-aspartic acid into the abnormal serum and urine proteins of multiple myeloma; studies of the interrelationship of these proteins. J Clin Invest 36:352–360.

Ou L, Duan D, Wu J, Nice E, Huang C. 2012. The application of high throughput siRNA screening technology to study host-pathogen interactions. Comb Chem High Throughput Screen 15:299–305.

Panley A, Mann M. 2000. Proteomics to study genes and genomes. Nature 405:837–846.

Parkinson T. 2002. The impact of genomics on anti-infectives drug discovery and development. Trends Microbiol 10(Suppl):S22–S26.

Pattanakitsakul SN, Rungrongcharoenkit K, Kanlaya B, Sinchaikul S, Noisakran S, Chen ST, Malasit P, Thongboonkerd V. 2007. Proteomic analysis of host responses in HepG2 cells during dengue virus infection. J Proteome Res 6:4592–4600.

Pauli EK, Schmolke M, Wolff T, Viennmann D, Roth J, Bode JG, Ludwig S. 2008. Influenza A virus inhibits type I IFN signaling via NF-kappaB-dependent induction of SOCS-3 expression. PLoS Pathog 4:e1000196.

Peng X, Li Y, Walters KA, Rosenzweig E, Lederer S, Aicher L, Proll S, Katze M. 2009. Computational identification of hepatitis C virus associated microRNA-mRNA regulatory modules in human livers. BMC Genomics 10:373.

Perry AK, Chow EK, Goodough JB, Yeh WC, Cheng G. 2004. Differential requirement for TANK-binding kinase-1 in type 1 interferon responses to toll-like receptor activation and viral infection. J Exp Med 199:1651–1658.

Pitteri SJ, Chrisman PA, Hogan JM, Luckey SA. 2005. Electron transfer ion/ion reactions in a three-dimensional quadrupole ion trap: reactions of doubly and triply protonated peptides with SO2+. Anal Chem 77:1831–1839.

Pritchard CC, Cheng HH, Tewari M. 2012. MicroRNA profiling: approaches and considerations. Nat Rev Genet 13:358–369.

Prodhom G, Bizzini A, Durussel C, Bille J, Greub G. 2010. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for direct bacterial identification from positive blood culture pellets. J Clin Microbiol 48:1481–1483.

Qi Y, Tu J, Cui L, Guo X, Shi Z, Li S, Shi W, Shan Y, Ge Y, Shan J, et al. 2010. High-throughput sequencing of microRNAs in adenovirus type 3 infected human laryngeal epithelial cells. J Biomed Biotechnol 2010:915980.

Rabilloud T. 2002. Two-dimensional gel electrophoresis in proteomics: old, old fashioned, but it still climbs up the mountains. Proteomics 2:3–10.

Rahman MM, McFadden G. 2011. Modulation of NF-kB signalling by microbial pathogens. Nat Rev Microbiol 9:291–306.

Ramanathan R, Jenial M, Ramagrit S, Xia YQ, Humphreys WG, Olah T, Korfmancher WA. 2011. It is time for a paradigm shift in drug discovery bioanalysis: from SRM to HRMS. J Mass Spectrom 46:595–601.

Rasheed S, Yan JS, Hussain A, Lai B. 2009. Proteomic characterization of HIV-modulated membrane receptors, kinases and signal-
ing proteins involved in novel angiogenic pathways. J Transl Med 7:75.

Bighetti PG, Castagna A, Antonucci F, Pinubelli C, Cecconi D, Camporotini N, Antonioli P, Astner H, Hamdan M. 2004. Critical survey of quantitative proteomics in two-dimensional electrophoretic approaches. J Chromatogr A 1051:3–17.

Rolen U, Kozheva V, Gasparjan N, Ova H, Winberg G, Kisseljov F, Masucci MG. 2006. Activity profiling of deubiquitinating enzymes in cervical carcinoma biopsies and cell lines. Mol Carcinog 45:260–269.

Rossi F, Gallina A, Milanesi G. 1996. Nef-CD4 physical interaction sensed with the yeast two-hybrid system. Virology 217:397–403.

Rottiers V, Naar AM. 2012. MicroRNAs in metabolism and metabolic disorders. Nat Rev Mol Cell Biol 13:239–250.

Rowan AG, Fletcher JM, Ryan EJ, Moran B, Hegarty JE, O’Farrelly R, Naar AM. 2012. MicroRNAs in metabolism and metabolic disorders. Nat Rev Mol Cell Biol 13:239–250.

Schena M, Shalon D, Davis RW, Brown PO. 1995. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270:467–470.

Schena M, Shalon D, Davis RW, Brown PO. 1996. A DNA microarray system for monitoring of 1000 genes. Proc Natl Acad Sci USA 93:10614–10619.

Schmid MB. 2001. Microbial genomics—new targets, new drugs. Expert Opin Ther Targets 5:465–475.

Schwahn AB, Wong JW, Downard KM. 2010. Typing of human and animal strains of influenza virus with conserved signature peptides of matrix M1 protein by high resolution mass spectrometry. J Virol Methods 165:178–185.

Seconil TP, Blethrow JD, Schwartz JC, Merrihew GE, MacCoss MJ, Swaney DL, Russell JD, Coom JJ, Zahroukov V. 2009. Dual-pressure linear ion trap mass spectrometer improving the analysis of complex protein mixtures. Anal Chem 81:7757–7765.

Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, Raoult D. 2009. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis 49:543–551.

Shapira SD, Gat-Viks I, Shum BO, Dricot A, de Grace MM, Wu L, Gupta PB, Hao T, Silver SJ, Root DE, et al. 2009. A physical and regulatory map of host-influenza interactions reveals pathways in H1N1 infection. Cell 139:1255–1267.

Sharma K, Tripathi S, Ranjan P, Kumar P, Garten B, Deyde V, Katz JM, Cox NJ, Lal RB, Sambhara S, et al. 2011. Influenza A virus nucleoprotein exploits Hsp40 to inhibit PKR activation. PLoS ONE 6:e20215.

Simons N, Rual JF, Lemmens I, Boxus M, Hirozane-Kishikawa T, Gato J, Dricot A, Hao T, Vertommen D, Legros S, et al. 2012. Host-pathogen interactome mapping for HTLV-1 and 2 retroviruses. Retrovirology 9:26.

Singaravelu B, Blais DR, McKay CS, Pezacki JP. 2010. Activity-based protein profiling of the hepatitis C virus replication in Huh-7 hepatoma cells using a non-directed active site probe. Proteome Sci 8:5.

Stasko D, Hoffmann SP, Kim KC, Fackler NL, Larsen AS, Drovetskaya T, Tham FS, Reed CA, Rickard CE, Boyd PD, et al. 2002. Molecular structure of the solvated proton in isolated salts. Short, strong, low barrier (SSLB) H-bonds. J Am Chem Soc 124:13969–13976.

Stears RL, Martinsky T, Schena M. 2003. Trends in microarray analysis. Nat Med 9:140–145.

Steuerwald NM, Parsons JC, Bennett K, Bates TC, Bonkowsky HL. 2010. Parallel microRNA and mRNA expression profiling of (genotype 1b) human hepatoma cells expressing hepatitis C virus. Liver Int 30:1490–1504.

Sudarsanam P, Iyer VR, Brown PO, Winston F. 2000. Whole-genome expression analysis of snf/swi mutants of Saccharomyces cerevisiae. Proc Natl Acad Sci U S A 97:3364–3369.

Supekova L, Supek F, Lee J, Chen S, Gray N, Pezacki JP, Schlaphard A, Schultz PG. 2008. Identification of human kinases involved in hepatitis C virus replication by small interference RNA library screening. J Biol Chem 283:29–36.

Suter B, Kittanakorn S, Staglar I. 2008. Two-hybrid technologies in proteomics research. Curr Opin Biotechnol 19:316–323.

Tafforeau L, Chantier T, Pradezynski F, Pellet J, Mangeot PE, Vidalain PO, Andre P, Rabourdin-Combe C, Lotteau V. 2011. Generation and comprehensive analysis of an influenza virus polymerase cellular interaction network. J Virol 85:13010–13018.

Tai AW, Benita Y, Peng LF, Kim SS, Sakamoto N, Xavier RJ, Chung RT. 2009. A functional genomic screen identifies cellular cofactors of hepatitis C virus replication. Cell Host Microbe 5:298–307.

Tan SL, Ganji G, Fauper B, Proll S, Katze MG. 2007. Systems biology and the host response to viral infection. Nat Biotechnol 25:1383–1389.

Tarao K, Fujiyama S, Ohkawa S, Miyakawa K, Tamai S, Hirokawa S, Masaki T, Tanaka K. 2005. Ursodiol use is possibly associated with lower incidence of hepatocellular carcinoma in hepatitis C virus-associated liver cirrhosis. Cancer Epidemiol Biomarkers Prev 14:164–169.

Tatomina R, Fujiyama S, Okawara S, Miyakawa K, Tamai S, Hirokawa S, Masaki T, Tanaka K. 2005. Ursodiol use is possibly associated with lower incidence of hepatocellular carcinoma in hepatitis C virus-associated liver cirrhosis. Cancer Epidemiol Biomarkers Prev 14:164–169.

Targett-Adams P, Boultant S, McLaughlan J. 2008. Visualization of double-stranded RNA in cells supporting hepatitis C virus RNA replication. J Virol 82:2182–2195.

Templin MF, Stoll D, Schrenk M, Tsaban P, Vohringer CF, Joho TO. 2002. Protein microarray technology. Trends Biotechnol 20:160–166.

Tisoncik JR, Behisle SE, Diamond DL, Korth MJ, Katze MG. 2009. Is systems biology the key to preventing the next pandemic? Future Virol 4:553–561.
To A, Bai Y, Shen A, Gong H, Umanoto S, Lu S, Liu F. 2011. Yeast two hybrid analyses reveal novel binary interactions between human cytomegalovirus-encoded virion proteins. PLoS ONE 6:e17796.

Tyers M, Mann M. 2003. From genomics to proteomics. Nature 422:193–197.

Wang J, Tan J, Zhang X, Guo H, Zhang Q, Guo T, Geng Y, Qiao W. 2010. BFA activates the NF-[kappa] B pathway through its trans-activator (BTas) to enhance viral transcription. Virology 400:215–223.

Waris G, Huh KW, Siddiqui A. 2001. Mitochondrially associated hepatitis B virus X protein constitutively activates transcription factors STAT-3 and NF-kappa B via oxidative stress. Mol Cell Biol 21:7721–7730.

Williams TL, Luna L, Guo Z, Cox NJ, Donis RO, Barr JR. 2008. Quantification of influenza virus hemagglutinins in complex mixtures using isotope dilution tandem mass spectrometry. Vaccine 26:2510–2520.

Wilson JA, Richardson CD. 2005. Hepatitis C virus replication escapes RNA interference induced by a short interfering RNA directed against the NS5b coding region. J Virol 79:7050–7058.

Wong KK, Engelman JA, Cantley LC. 2010. Targeting the PI3K signaling pathway in cancer. Curr Opin Genet Dev 20:87–90.

Wu HL, Huang LR, Huang CC, Lai HL, Liu CJ, Huang YT, Hsu YW, Lu CY, Chen DS, Chen PJ. 2005. RNA interference-mediated control of hepatitis B virus and emergence of resistant mutant. Gastroenterology 128:708–716.

Yee J, White RE, Anderton E, Allday MJ. 2011. Latent Epstein-Barr virus can inhibit apoptosis in B cells by blocking the induction of NOXA expression. PLoS ONE 6:e28506.

Yerly S, Kaiser L, Race E, Bru J, Clavel F, Perrin L. 1999. Transmission of antiretroviral-drug-resistant HIV-1 variants. Lancet 354:729–733.