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Opportunities in proteomics to understand hepatitis C and HIV coinfection

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Abstract

Antiretroviral therapy has significantly reduced morbidity and mortality associated with HIV infection. However, coinfection with HCV results in a more complicated disease course for both infections. HIV infection dramatically impacts the natural history of chronic liver disease due to HCV. Coinfected patients not on antiretroviral therapy for HIV develop liver fibrosis and cirrhosis at a faster rate, clear acute infection less commonly and respond to IFN- α -based therapy for chronic infection less often than HCV-monoinfected patients. The interaction between these two viruses, the immune system and the fibrotic machinery of the liver remains incompletely understood. In this review, we discuss recent advances in proteomics as applied to HCV and HIV and highlight issues in coinfection that are amenable to further discovery through proteomic approaches. We focus on clinical predictors of liver fibrosis and treatment outcome as these have the greatest potential clinical applicability.

Keywords

coinfection; fibrosis; hepatitis C; HIV; proteomics; treatment prediction

HCV infection & liver fibrosis

Viral hepatitis due to HCV causes substantial morbidity and mortality. HCV-associated deaths are now comparable in number to HIV-associated deaths in the USA [1]. Up to a third of patients infected with HIV harbor concomitant HCV infection, with a high prevalence in injection drug users [2–4]. The natural history of HCV-induced liver disease is affected in several ways by untreated HIV coinfection, most notably with faster progression of liver fibrosis and cirrhosis compared with HCV-monoinfected patients (Table 1). Potential mechanisms for accelerated disease include increased gut microbial translocation

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leading to increased stellate and Kupffer cell activation, generalized immune activation from HIV and direct infection of hepatic stellate cells by HIV (shown *in vitro*) [5–7]. The ability to predict the rate of liver fibrosis would have clinical utility for treatment and transplant decisions as well as reduce the need for invasive diagnostic procedures and their associated risks and limitations.

Proteomics

Proteomics was originally described as the large-scale analysis of the function of genes [8]. The field has continued to evolve into both basic and applied sciences. Understanding protein expression, interactions and post-translational modifications has provided insight into the biology of disease and has produced mechanistic information regarding pathogenesis. Clinical proteomics holds promise for the discovery of biomarkers that may provide information on disease risk, responses to therapy and prognosis.

The basic and clinical applications of proteomics share similar technologic approaches with a high reliance on simplifying complex mixtures by sample fractionation and the use of diverse mass spectrometry (MS) platforms [9]. Multiple technical options are available to delineate protein expression based on two fundamental approaches (reviewed in [10]). The bottom-up approach simplifies complex samples by proteolytically digesting proteins prior to MS analysis. The resulting MS-derived peptide masses and sequences then serve as surrogate markers for their respective intact proteins. A major challenge with this approach is the computational analysis of the multiple peptide sequences that are generated using database searches and comparing these sequences with predicted *in silico*-generated fragmentation patterns. The alternative strategy to evaluate protein expression is the top-down method used to analyze intact proteins, their fragments and post-translational modifications. The pre-analytic separation of a protein mixture prior to MS analysis with this approach has its own set of unique challenges (reviewed in [10]). Difficulty in separating and fragmenting large proteins has restricted most top-down experiments to single proteins or restricted protein mixtures [10].

The workflow to discover and develop novel protein biomarkers is relatively analytic-platform independent [11]. However, identifying and characterizing one or more proteins that can reliably serve as a marker of health or disease requires substantial validation based on large numbers of samples and assays that are accurate, have appropriate throughput and are economically feasible [11].

HCV & proteomics

Recent work in HCV proteomics has utilized either liver tissue or serum to correlate protein expression with the rate of fibrosis in patient cohorts with defined outcomes in order to develop predictive algorithms (Table 2). Diamond *et al.* examined a cohort of liver transplant patients monoinfected with HCV who had accelerated liver fibrosis [12]. Using liquid chromatograph-MS of liver tissue obtained 6 and 12 months post-transplant they defined 250 proteins associated with rapid liver fibrosis. The functional annotation of these proteins showed an enrichment of proinflammatory proteins and decreased expression of antioxidant and hepatoprotective proteins [12]. The serum proteomic profile of a different patient cohort also showed correlation of impaired antioxidant proteins in patients with more rapid post-transplant liver fibrosis [12]. Previous work by these authors used a cross-sectional analysis of liver protein expression by tandem MS and identified 210 proteins associated with oxidative stress whose expression correlated with the stage of fibrosis [13]. Yang *et al.* studied serum of a cohort of HCV-infected patients with known degrees of hepatic fibrosis using isobaric tags for relative and absolute quantification and 2D difference in gel electrophoresis [14]. They identified a small number of proteins whose expression

correlated with fibrosis including two potential biomarkers (complement C4-A and inter- α -trypsin inhibitor heavy chain H4) [14]. The data obtained from these studies exemplify the potential of proteomic strategies to discover molecular signatures associated with the rate of liver fibrosis and await validation in other patient cohorts. To date, no similar studies have focused on patients infected with both HIV and HCV.

Predicting outcomes of hepatitis C treatment

Patients infected with HIV are less likely to respond to IFN- α -based therapy for treatment of chronic HCV relative to HCV-monoinfected patients, with longer treatment duration sometimes required and a lowered chance of achieving a sustained virologic response (SVR) (Table 1). Proteomic approaches have attempted to define molecular signatures in serum that will predict treatment outcome in HCV monoinfection. Using liquid chromatograph-MS of pretreatment serum, Patel et al. identified three proteins (vitamin D binding protein, α 2 HS glycoprotein and complement C5) whose expression pretreatment correlated with the likelihood of achieving SVR [15]. Notably, this protein signature performed well when applied to an independent cohort of patients [15]. Devitt et al. studied serum obtained 24 h after starting therapy and used surface enhanced laser desorption ionization time of flight (SELDI-TOF) MS to identify 70 serum peaks that distinguished nonresponders from patients who achieved SVR [16]. Twenty of these peaks were used in an algorithm that had 87% predictive accuracy in identifying non-responders when applied to the original cohort [16]. Similarly, Paradis et al. used SELDI-TOF analysis of pretreatment serum to develop a predictive model of treatment outcome using protein peaks, fibrosis stage and viral genotype that showed 81% accuracy when applied to an independent cohort [17]. The SELDI-TOF platform is a semiquantitative tool for the discovery of protein expression patterns and for hypothesis generation. It provides a rapid assessment of protein expression in biologic samples and serves to incentivize further detailed analysis of its proteomic signatures. However, assay variability and the inability to directly identify protein peaks with SELDI-TOF precludes its clinical use currently (Table 2).

Efforts have also focused on analysis of the blood proteome of patients infected with HIV relative to uninfected patients, analyzing either total peripheral blood mononuclear cells or isolated CD4 lymphocytes [18,19]. The first plasma study in coinfecting patients analyzed pooled samples from HIV/HCV-coinfecting patients relative to HCV- and HIV-monoinfecting patients and healthy controls, and identified unique protein profiles in the four groups [20]. The majority of identified proteins were upregulated in all three groups of infected patients relative to controls with a few downregulated in the coinfecting population. Two notable categories of upregulated proteins were apolipoproteins and components of the complement pathway. Details of the multiple immune and inflammatory processes associated with these differentially expressed proteins and the contribution of treatment responses to their expression are unknown. Proteomics has yet to be applied towards predicting HCV treatment outcome in patients infected with HIV.

HCV viral proteomics

In addition to clinical predictive applications, proteomic approaches have been used to query fundamental questions about the pathogenesis and intracellular effects of HCV. The Huh-7.5 cell line, which is permissive for infection with the full length HCV replicon, has been extensively probed to define cellular proteome perturbation by HCV (reviewed in [21–24]). As an example, using a combination of methods including 2D electrophoresis, MS and stable isotope labeling by amino acids in cell culture, 150 proteins were differentially altered by HCV infection. Many of these proteins are involved in protein trafficking and cell signaling, and some were associated with distinct changes in subcellular localization and

enrichment in lipid rafts [25]. These data suggest that HCV proteins, like other viral proteins, show a predilection for interacting with human proteins that have multiple potential protein interactions (i.e., hubs) and proteins that are central to many pathways (i.e., bottlenecks) [26–28]. By combining known viral–host protein interactions derived from hypothesis-driven research and combining data obtained from blind proteome screening approaches, it is possible to create a theoretical network or interactome that encompasses the protein connectivity between virus and host [27,29]. These networks can be used to direct targeted therapeutics and drug design.

Challenges

Numerous challenges remain given the complexity of nature, patient-to-patient variability, and technical and computational limitations of biomarker discovery (Table 2). The large size of the human proteome (estimated to be several million different proteins), variable subcellular localization and difficulty identifying less abundant proteins remain formidable challenges [30–33]. A substantial number of protein peaks obtained during MS experiments are not identifiable using current databases, and many proteins predicted to exist based on the human genome have not been observed in *ex vivo* MS samples [34,35]. Further challenges arise as many proteins do not have clearly identified functions, lack reagents for further study, and cannot be amplified; thus, validating function and expression level remains difficult, as does explaining contribution to biologic phenomena. Finally, thus far, proteomics suffers from a lack of spatial resolution, with most experiments based on whole-tissue extraction rather than cell type-specific expression [36].

As such, proteomics, and in a broader sense systems biology, are currently driven more by organization and management of large pools of data and less by understanding of biological significance and mechanism (reviewed in [37]). Sound experimental design and statistical approaches to avoid false positive discovery are vital, as is validation of findings in independent cohorts of patients [38–40]. A variety of open platforms to help analyze proteomic data exist to help the researcher but all have their own limitations and require expertise in bioinformatics [41].

Future applications & perspective

The application of proteomics is a promising approach to the study of HCV and HIV coinfection, however, thus far, few studies have examined patients infected with both viruses. Examples of issues in the field of coinfection that are amenable to proteomic inquiry are detailed in Box 1. The most practical application of proteomics to predict both disease course and treatment response now has firm precedence in HCV mono-infection and use in HCV/HIV coinfection should follow. As HCV treatment evolves towards IFN- α free regimens, proteomic signatures that are associated with treatment response may become more apparent. Identification of protein(s) that could not only predict but also guide duration of therapy would be ideal for HIV/HCV-coinfected subjects, who as a group experience more relapse and lower rates of SVR compared with HCV-mono-infected patients treated with standard therapy.

Box 1

Questions in HIV and HCV coinfection amenable to proteomic inquiry

- Can we develop a proteomic signature that accurately predicts clearance of HCV in patients undergoing treatment with directly acting antivirals?

- Can we develop a proteomic profile that consistently is able to predict immediate and long-term relapse of HCV in patients receiving directly acting antivirals?
- What are the peripheral markers that correlate with extent of liver disease and may facilitate prognosis? (Goal: ability to prognosticate without needing liver biopsy.)
- Can a distinction be made between protein expression changes that are associated with viral clearance (i.e., response markers) versus proteins that actually mediate HCV clearance and cure?
- Are there unique proteomic markers for disease comorbidities such as diabetes and obesity?
- With emergence of directly acting antivirals and potential for viral resistance, can proteomics be applied to detect specific viral amino acid differences associated with resistance?
- Can proteomics be used to identify and track HIV and HCV viral quasispecies?
- Can a meaningful ‘interactome’ be created that incorporates HCV, HIV and host proteins even though these viruses may not infect a common cell population?

The future of HCV treatment will involve more consistent, reproducible and simpler diagnostic and prognostic tests with advances in individualized patient therapy. HIV coinfection as well as concomitant use of antiretrovirals will require consideration in treatment and prognostic algorithms for HCV. Proteomics holds great promise for biomarker discovery, however, additional validation is still required to advance present day proteomic tools to meet future needs. Before these techniques can be used clinically, several important factors that need to be addressed include variability of analyte (serum, plasma and tissue), operator variability, automation of assays, development of high throughput platforms for quick and easy results, and development of cost effective platforms that are affordable for routine clinical use.

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Executive summary

HIV & HCV coinfection

- Coinfection with hepatitis C and HIV results in more progressive liver disease than infection with hepatitis C alone.
- A third of HIV-infected patients in the USA are also infected with hepatitis C.
- Mechanisms of enhanced liver fibrosis in coinfection are poorly understood.

Treatment response

- Proteomics has been applied in hepatitis C monoinfection to predict rates of fibrosis using liver biopsy samples or patient serum and to identify serum markers that are associated with a likelihood of achieving treatment success for hepatitis C using IFN- α -based therapy.

Proteomics applied to HCV fibrosis & challenges

- Challenges to the application of proteomics include patient variability, technical challenges to detect low-abundance proteins, and informational challenges to verify function and mechanism of observed changes.

Future perspective

- Application of proteomics to HIV/HCV coinfection is a promising area for future study of disease prognosis, treatment response and viral pathogenesis.

Table 1

HCV disease factors affected by HIV coinfection.

Feature	Effect of HIV infection	Impact	Ref.
Prevalence	A third of HIV patients are also HCV infected	Increased burden of disease	[2–4]
Transmission	Increased transmission of HCV	Increased prevalence	[42,43]
Resolution of acute HCV	Decreased rate of resolution	Increased HCV chronicity	[5,44,45]
Development of HCV antibodies	Lower rate than HCV-monoinfected individuals	Higher rate of false negative tests	[46,47]
Liver fibrosis progression	Higher rate in untreated HIV subjects	More cirrhosis, HCC and death from liver disease	[5–7,48–52]
Liver enzyme elevations	Higher with use of ART, can be medication or IRIS related	Increased morbidity, possibly increases liver disease	[53–57]
HCV viral load	Increased in HIV-infected subjects, can increase with use of ART	More difficult to treat	[58–61]
Response to HCV treatment	Lower rates of SVR, slower viral response, more anemia and neutropenia	Lower cure rates, increased duration of treatment	[62,63]
Mortality	Increased in HIV-infected subjects	Major cause of non-AIDS mortality in western world	[49,64]

ART: Antiretroviral therapy; HCC: Hepatocellular carcinoma; IRIS: Immune reconstitution inflammatory syndrome; SVR: Sustained virologic response.

Table 2

Proteomic platforms as applied to HIV and HCV coinfection.

Platform	Description	Advantages	Disadvantages	Ref.
LC-MS	Classical platform; analyzes mass spectra of protein fragments	Gold standard; can identify protein peaks associated with outcome	Labor intensive, instrumentation and training required, generates large amounts of data to process, protein ID often difficult, abundant proteins may impact results	[12,13,15,19,20,25]
iTRAQ-DIGE	Uses electrophoresis to separate abundant proteins	Ideal to remove abundant proteins	Not useful for larger proteins, protein ID is required	[14,20]
SELDI-TOF	Separation based on microchips that bind to proteins with opposite properties	Can identify protein peaks associated with outcome	Labor intensive, instrumentation and training required, generates large amounts of data to process, protein ID very difficult, abundant proteins may impact results	[16,17]
SILAC	Isotope labeling to track or identify proteins. Ideal for <i>in vitro</i> and <i>in vivo</i> protein ID in animal models	Useful to identify methylation sites, protein substrates and subcellular proteomics	Limited application in humans	[13,25]

iTRAQ-DIGE: Isobaric tags for relative and absolute quantification and 2D difference in gel electrophoresis; LC-MS: Liquid chromatography–mass spectrometry; SELDI-TOF: Surface enhanced laser desorption ionization time of flight mass spectrometry; SILAC: Stable isotope labeling by amino acids in cell culture.