HIV-1 Infection and Genomics: Sorting Out the Complexity

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(See the article by Li et al., on pages 572–82.)

Since the first complete sequencing of the human genome demonstrated that there are ∼30,000 genes, rather than the 100,000 or more that had been hypothesized previously, the next steps in this remarkable field of biomedicine have begun to be explored. These include the use of genomics to understand both the function and interplay of the products of each of these genes, as well as their interactions with various cellular microenvironments [1, 2]. Genomics is the study of whole sets of genes or gene products, rather than the analysis of individual genes. Microarrays involve single-assay studies of many genes or gene products, using interrogation of the gene or gene products in a solid state. This usually includes the use of scanning and imaging devices and of software designed for computational analysis of the raw data [1].

Recent data help us understand the interactions of human lentiviruses, especially HIV-1, with cellular gene products using functional genomic analyses. The first series of studies have used HIV-1 infection of cell lines or isolated peripheral blood mononuclear cells in vitro to demonstrate in time-course assays the effects of infection on the expression of cellular gene products. Using micro- or macroarray gene chips, thousands or tens of thousands of mRNAs and the effects on these gene products that lead to decreased, increased, or unaltered expression have begun to be elucidated [3–6]. In these first studies, microarray analysis at low multiplicity of HIV-1 infections or in heterogeneous target cell populations was analyzed. Unfortunately, the results of these studies are difficult to interpret, because nonsynchronized infection can lead to confounding changes in gene expression over time. A recent study has helped shed some clarity on this already complex series of initial studies [7]. In that article, high-titer virus was used to infect various isolated CD4+ T cell lines, and the levels of expression of >4600 cellular RNA transcripts were analyzed. Alterations in several classes of gene products associated with the cell-cycle G2 arrest generated by HIV-1, in response to the viral regulatory protein Vpr, were demonstrated [8]. A variety of transcription factors were increased, as well as series of enzymes involved with cholesterol biosynthesis, which is interesting in light of the importance of cholesterol and lipid rafts that has recently been shown during entry of HIV-1 into cells and morphogenesis of viral progeny [9]. Genes involved with cell division and transcription and a family of DEAD box protein–RNA helicases and genes involved with translation and RNA splicing were up-regulated [7]. These initial studies confirmed previous demonstrations of interactions of isolated viral proteins with and viral infection of host cells and also elucidated other, potentially new, cellular pathways perturbed by HIV-1 infection.

Sorting out these data is complicated enough, but into this mix now comes an exciting and superbly performed study by Li et al. [10] at the Haase laboratory, in which functional genomics was used to evaluate the effects of antiretroviral therapy on HIV-1 infection of lymphatic tissue. This study is unique in that it not only evaluates patients in cross-section, examining lymph nodes at 1 time point, but actually studies the same patients before and after treatment. Previous data have shown that antiretroviral therapy in general, and highly active antiretroviral therapy (HAART) in particular, have profound effects and not only decrease viremia but also alter T cell distribution and function [11–13]. The data in the article by Li et al. [10], which is published in this issue of The Journal of Infectious Diseases, are important, because they suggest molecular mechanisms that may now begin...
to explain the decreases in T cell activation and changes in T cell distribution between the periphery and lymphoid tissue that occur soon after the initiation of virologically suppressive therapy [14].

The study by Li et al. [10] evaluated ~200 “treatment-responsive” genes before and after antiretroviral therapy and demonstrated that most of the alterations in lymphoid tissues were in genes categorized as affecting cell trafficking, tissue repair, and innate immunity. These are each important, because they suggest areas of HIV-1 pathogenesis that may be amenable to novel therapies. First, gene products involved with innate immunity, including interferon-γ and others, may play a critical role in both early and chronic infection by decreasing but not eradicating HIV-1 infection [10, 15]. Recent data suggest [9], although this is very controversial [16, 17], that α-defensins may be important in the production of the long-term nonprogression states and may be involved in the innate immune response to HIV-1. Other studies, in which techniques such as those described in the article by Li et al. [10] were used in a wide variety of treatment-naive individuals and individuals receiving long-term treatment during HIV-1 infection, will be necessary to evaluate the potentially prominent role of innate immunity during HIV-1 infection. Nonetheless, it is very difficult to be sure that the alterations demonstrated in a functional genomic analysis really have a substantial antiviral effect and do not represent a series of HIV-1–induced increases in mRNA expression that are a relic of our evolutionarily persistent innate immune system and may not be substantially active against this viral pathogen. Nonetheless, the fact that the human genome is ~10% retrotransposons, which suggests that we have been attacked by multiple retroviral agents throughout our 1.7 billion years of eukaryotic evolution and development, suggests that innate immune responses may be important in modulating the host-virus interaction for various primate retroviruses.

Decreases in immune activation gene products were demonstrated in this study, and these findings are not unexpected. Untreated HIV-1 infection leads to immune activation, which may yield some of the symptoms involved with chronically untreated HIV-1 disease, especially after substantial CD4+ T lymphocyte decline [13]. Nonetheless, it is important to catalogue and quantify the different immune activation gene products that are affected by HAART.

Down-modulation of immune activation parameters after viral suppression is an important phenomenon [13, 18] that was well demonstrated in the functional genomics study by Li et al. [10]. The authors suggest that these findings may allow the development of new and targeted therapies to decrease the immune activation observed in patients with ongoing viral replication. This would be significantly more attractive than using generalized immune-suppressing drugs, such as corticosteroids, and, thus, functional genomics may allow us to pick and choose targets and attack not only the virus but the immune activation that it induces.

One cautionary note is that although the effects on cellular gene expression noted before and after antiretroviral therapy are likely to be mainly the result of decreases in viral replication, the direct effects of the antiretroviral agents themselves (especially protease inhibitors) must also be considered. Protease inhibitors have been demonstrated to directly alter antigen presentation via changes of the proteasome and ubiquitination pathways [19].

Quantitative expression changes in tissue repair genes [20], also demonstrated in the study by Li et al. [10], were somewhat disjointed; some increased and others decreased after therapy. A number of empirical hypotheses could be developed to explain this, but further work is necessary to determine the importance of each of the pathways. It was interesting to note that the activity of the genes encoding metalloproteinases increased with antiretroviral therapy. Although this may be important in mediating the tissue remod-
targets for development of small-molecular inhibitors of the virus or drugs to interdict in select virus–host cell interactions. One of the next important stages in understanding infection is sifting through important cellular cofactors involved with infection and production of viral progeny. Sometimes functional genomics is useful, but it is important to recognize that, because posttranscriptional effects may be critical in certain virus–host cell interactions and because absolute quantities of a specific viral mRNA may not illustrate all of these critical interactions, we will also have to approach HIV-1 infection using proteomics [2]. This is the detailed analysis of large numbers of protein products themselves, rather than the mRNA analyses evaluated in certain functional genomic approaches.

Recent studies of HIV-1 and the host cell have demonstrated such new and novel findings as the interactions of the previously enigmatic lentiviral regulatory protein, Vif, and its now well-described interaction with the host viral protein CEM15 or APOBEC3G, which has been shown to alter hypermutation of the viral genome [23–26]. This is an excellent example of how analyzing clear virus–host cell protein interactions may lead to a previously unknown antiviral target. Our laboratories have also used functional genomics and microarray technology to sort out the effects of HIV-1 viral proteins, such as Vpr, that lead to a specific pathway of programmed cell death (apoptosis) in human neurons and, therefore, possibly identify a key player in HIV-1–induced encephalopathy [27].

The effects of ongoing viral replication and free gp120 expression in the peripheral blood plasma of HIV-1–infected individuals have been shown to profoundly alter a cascade of cellular signaling proteins in both nonproliferating and proliferating target cells for HIV-1 [28]. HIV-1 envelope genes may modulate the cellular microenvironment to allow CD4+ T cells that are not fully activated to become productively infected. This molecular mechanism may explain the phenomenon of HIV-1 expression and reservoir sites in semi-quiescent T lymphocytes in humans and simian immunodeficiency virus (SIV)–infected monkeys [22]. A recent article that evaluated gene expression and viral production in latently infected resting CD4+ T lymphocytes in viremic versus aviremic HIV-1–infected individuals demonstrated that during viremia a variety of cellular gene products, as assayed by microarray analyses, including genes involved with transcription, mRNA processing, and protein and vesicular transport, were significantly up-regulated in viremic patients, compared with patients who remained aviremic [29]. This suggests that, depending on the cell type evaluated in patients who are and are not receiving antiviral treatment, a series of somewhat differing data sets may be obtained. Recent data, however, have suggested that there may be differing levels of virologic suppression in patients receiving HAART, even when the plasma virus load is <50 copies/mL [30]. Thus, subgroup analyses may be required in functional genomic studies.

Evaluating cell types other than the lymphatic tissue and peripheral blood cells will also be important when functional genomic analysis is used. We have demonstrated that HIV-1 Vpr dramatically altered bone marrow macrophages, increasing their activation state and their phagocytic ability [31]. Further studies in bone marrow in which functional genomics is used may help investigate the hematopoietic dysfunction that occurs in many untreated and some antiretroviral-treated HIV-1–infected individuals. In addition, the effects of various HIV-1 cofactors, including use of ethanol and other substances of abuse (e.g., cocaine and opiates), should also be evaluated using functional genomic methods. We have recently demonstrated that ethanol augments HIV-1–induced apoptosis of central nervous system microvascular endothelium [21]. Ongoing studies using microarray technology will likely be able to analyze the complex pathways involved with this and other exogenous cofactors of HIV-1 infection. Similarly, the HIV-1 regulatory protein Nef, which increases HIV-1 replication and immune activation and robustly interacts with various cellular protein kinases, is likely to profoundly regulate cellular mRNA expression in many types of HIV-1–infected cells [32].

It is not clear that functional genomics will be able to identify which effects in the intact living body are associated with the virus or the direct inhibition of viral infection and which are “downstream” alterations in cellular gene products. It is becoming clear that HIV-1 infection leads to a profoundly complex series of crosstalk mechanisms between infected and noninfected cells [33]. A recent study has demonstrated that HIV-1–infected macrophages actually alter CD4+ T cells to allow progression of HIV-1 infection and reservoir disease by cytokines that first interact with B cells and then in turn communicate with and alter the T cell activation state [34]. This is an example of a process that would be difficult to elucidate using functional genomics alone.

The effects of certain cellular stimulatory agents, such as OKT3, a murine monoclonal antibody against the T cell receptor, which have been studied in attempts to purge reservoirs of latent HIV-1 in humans [35, 36], will also be important to study using genomic technology. We have recently demonstrated that a novel agent that increases HIV-1 expression from proviral latency, prostratin, yielded surprising genomic data in treated cells, in which a pathway different from that of other, more commonly used T cell stimulatory agents, was demonstrated [37]. These molecular approaches can be used to explore not only the effects of antiretroviral therapy but specific interactions of the virus and its cofactors with mixed-cell and single-cell populations during HIV-1 infection.

This is complex enough—but there are more flies in the ointment. Functional genomic analysis of mRNAs does not evaluate
posttranscriptional alterations (this would require proteomic analysis), but it has been shown that many feedback mechanisms of cellular control are not caused by proteins. New data on small interfering RNAs and RNA interference have set up a true paradigm shift in the understanding of cellular control and, therefore, the effects of viral pathogens on human cells [38]. It appears that a significant quantity of cellular control is due to these small, nongene RNAs, which are part of what has been called the “micro-RNA world” and make up more of the human genome than do genes. If we expect to understand the whole picture by using functional genomics, with or without proteomics, we are still in for a further surprise with regard to the complexity of the control mechanisms and information transfer in the human cell and exploring the environmental agents that perturb it. Thus, functional genomics will not be a panacea. This being said, functional genomics can give us clinically relevant clues to (1) viral protein–host cell interactions, both descriptive and mechanistic; (2) the effects of therapies on HIV-1 infection and their direct and/or indirect interactions with the host immune- and nonimmune-based cells; and (3) new targets for antiviral agents and downstream targets for therapies to interfere in virus-induced pathogenetic processes (e.g., immune activation).

A very recent study has even shown that gene expression profiling can be used to evaluate the alterations in intestinal cell cycle–specific moieties and growth factors after SIV infection [39]. Thus, genomics will be very useful in dissecting out the effects of primate lentiviruses in animal models of HIV-1 disease.

In summary, the article by Li et al. [10] from the Haase laboratory is an excellent initial foray into the field of human functional genomics as it applies to HIV-1 infection. I predict that studies such as this will be critical in understanding the remarkable complexity of HIV-1 interactions with human cells, in particular, and the intact human organism, in general. Nevertheless, interactions of various cell types during HIV-1 infection and, therefore, cellular cross-talk will remain difficult to fully understand. The present study begins the trek toward important new targets for molecular inhibition of HIV-1 disease, possibly, as suggested, anti-inflammatory agents that do not directly target HIV-1 replication but alter its proinflammatory effects on certain human cells. Nevertheless, even after use of these remarkably potent molecular techniques has become common in translational HIV-1 research, we will continue to be surprised by how such a relatively simple lentivirus, with only 9.7 kb of information, can lead to such an intertwining network of interactions in the human host.

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