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Roseomics: a blank slate

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Abstract

Recent technological advances have led to an explosion in the system-wide profiling of biological processes in the study of herpesvirus biology, herein referred to as "-omics". In many cases these approaches have revealed novel virus-induced changes to host cell biology that can be targeted with new antiviral therapeutics. Despite these successes, -omics approaches are not widely applied in the study of roseoloviruses. Here we describe examples of how -omics studies have shaped our understanding of herpesvirus biology, and discuss how these approaches might be used to identify host and viral factors that mediate roseolovirus pathogenesis.

Keywords

Human Herpesviruses; Roseoloviruses; HHV-6A; HHV-6B; HHV-7

Introduction

Over the past several years, advancements in high-throughput systems biology approaches and technologies have resulted in major vertical achievements in the field of molecular biology. For the purpose of this review, omics refers to a systems biology approach for defining the components of a biologicial system and their interactions with one another. Common examples of -omics approaches include (but are not limited to) functional genomics, transcriptomics, metabolomics, and proteomics (reviewed in (1). These approaches have been successfully applied to several herpesviruses including human herpesvirus 8 (HHV8; also known as Kaposi's sarcoma-associated herpesvirus or KSHV), herpes simplex virus (HSV), human cytomegalovirus (HCMV) and murid herpesvirus 4 (MuHV4; also known as murine gammaherpesvirus-68 or MHV-68) which are discussed below. In each case these studies have vastly expanded our understanding of the unique

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biology of herpesvirus-infected cells. The unbiased nature of -omics studies has led to a wealth of innovative, testable hypotheses and identified novel virus-induced changes to host signaling pathways that impact viral replication and subsequent pathogenesis. While genomic and transcriptomic studies have identified new herpesvirus coding regions and transcripts, metabolomics and proteomics studies have defined novel interactions between viruses and host metabolic pathways and protein complexes. While each of these approaches alone provides a wealth of information, integrating the various -omics approaches generates a holistic understanding of the host-pathogen interactome.

In contrast to the other herpesviruses, few if any -omics approaches have been applied to the study roseoloviruses, comprised of human herpesviruses 6A, 6B and 7. It is now feasible to apply most, if not all, of the standard -omics approaches to the study of roseoloviruses. In this review we highlight opportunities for -omics approaches to rapidly advance our understanding of rosoleovirus biology and describe critical unmet research needs where –omics approaches should prove useful.

Functional Genomics

Functional genomics encompasses the fields of genome sequencing, comparative analysis of related genomes, and screening of phenotypic changes within an organism upon disruption of a candidate open reading frame (ORF). Within a decade after the first roseolovirus genome was discovered in 1986 (2), representative genomes of HHV-6A, HHV-6B and HHV-7 were sequenced (Accession #s NC_001664, AF_157706 and U43400 respectively). Since the original sequencing of these viruses, additional full-length genomes were reported (3–7). Due to distinct characteristics of these viruses including cellular and tissue tropism, genomic arrangement, peptide coding capacity and subsequent pathogenesis (8, 9), each of these roseoloviruses are classified as separate viruses (10). These studies were the first examples of an -omics approach in the study of roseoloviruses. *In silico* analyses revealed roseolovirus coding regions conserved across herpesvirus families, conserved amongst roseoloviruses, and most importantly, those unique to each roseolovirus (3–5). For many roseolovirus genes, putative functions were inferred based on homology to orthologous herpesvirus genes of known function. In addition these analyses allowed for focus on novel ORFs that may be involved in distinct roseolovirus pathogenesis. These studies provided the first insights into the organization and potential functional capacity of roseoloviruses.

Subsequent functional genomics studies have predominantly employed reverse genetics approaches, identifying phenotypic changes resulting from mutation or deletion of a specific viral ORF. These studies have almost exclusively focused on HHV-6A, as it is the only roseolovirus genome that has been cloned into a bacterial artificial chromosome (BAC) (11) and is therefore applicable to genetic manipulation. Examples of reverse genetic approaches in roseoloviruses include defining the role of viral glycoproteins in replication (11), and confirming the role of homologous genes conserved across the herpesvirus family (12).

Several additional functional genomics approaches have been successfully used in other herpesviruses and are amenable to the study of roseoloviruses. While only a handful of roseolovirus genomes have been sequenced, multiple full-length genome sequences are

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available for many other human herpesviruses. Comparison of the coding capacity of multiple strains has led to a better understanding of both virus evolution and replication (13). An excellent example is HCMV, where clinical strains were found to contain approximately 15 kb of DNA that is deleted or inverted during laboratory passage thereby altering the growth and tropism of these strains (14). Only upon additional sequencing of clinical isolates was it found that these alterations resulted in the loss-of-function of cytomeglaovirus genes essential for viral latency (15). Presumably roseoloviruses display a similar degree of heterogeneity, however this hypothesis has not been tested. The widespread availability of next generation sequencing coupled with advances in genome analysis and assembly makes this an attractive area for future roseolovirus studies.

Forward genetics approaches have also proven useful in defining novel functions for herpesvirus genes. Forward genetics refers to the process of screening random mutants for a specified phenotype. Subsequent genotyping of the selected variants then reveals the underlying gene (or genes) controlling the phenotype. Many herpesviruses including HCMV, HSV1, and MuHV4 have used genome-wide forward genetics studies to characterize viral genes. Libraries of expression vectors containing herpesvirus ORFs have proven useful in forward genetic screens to identify novel functions for viral genes, including antagonism of the host antiviral response (16) and manipulation of the cell cycle (17). In addition, global mutagenesis approaches have identified genes in MuHV4 (18), MCMV (19) and HCMV (20, 21) needed for efficient virus replication. A global determination of the complement of HHV-6A coding regions required for replication should be employed to identify novel targets for antiviral drugs. Similarly, a comprehensive collection of expression vectors for roseolovirus genes would allow for the identification of viral genes that contribute to unique aspects of the roseolovirus life cycle, for example genome integration. While HHV-6A, -6B and HHV-7 genomes have each been annotated, the field is hampered by a lack of BAC constructs for HHV-6B and HHV-7, thus making the essential development of forward genetics screens currently unavailable.

Transcriptomics

The most common, and often first employed, analysis of viral infection is monitoring changes in both viral and cellular transcription. High throughput qualitative profiling of transcript changes often relies on microarray technologies where one can monitor literally thousands of cellular transcripts or complete annotated viral transcripts in a single experiment. This technology has been used successfully to study the kinetics of both HHV-6A and -6B transcription (22, 23). This powerful resource is important for identifying the timing and relative levels of transcription from the viral genome. However, this methodology lacks absolute quantification and is biased towards regions of the genome that are previously known to be transcribed, as predefined probe sets are used as bait for transcripts. Next generation sequencing approaches can be used to address these deficiencies. These approaches allow for the unbiased profiling of both cellular and viral transcripts at saturating levels, thereby providing insight into both the absolute levels of transcripts and also transcript structure (reviewed in (24). While RNA based deep sequencing has identified roseolovirus transcripts in a few patient samples (25, 26), and RNA based deep sequencing has been used to identify small non-coding RNAs encoded by

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HHV-6B (27), a targeted approach to define roseolovirus mRNAs in a controlled infection has surprisingly not yet been performed. Such an approach would likely refine our understanding of the temporal expression of rosoleovirus genes, and potentially identify novel coding regions of the viral genome.

Alternative approaches to next generation RNA sequencing have been used with success for other herpesviruses including reverse transcriptase coupled real time quantitative PCR (RT-qPCR) whole genome panels (e.g. (28)) as well as the use of tiled arrays (e.g. (29)). The use of a tiled array for the related herpesvirus MuHV4 allowed for highly reproducible quantitative and qualitative resolution (20nt) of viral transcription during both lytic replication and reactivation from latency (29). In this study, not only were the kinetics of viral transcription assessed but the authors identified a previously undefined ORF that was not characterized by *in silico* analysis, underscoring the need for multiple –omics approaches for roseolovirus studies. Each of these above mentioned technologies are readily applicable and necessary for understanding the viral lifecycle of roseoloviruses and thus should be prioritized.

Proteomics

Proteomics concerns the large-scale study of structure, modification, function and abundance of proteins. Once a rarity, proteomics approaches have become increasingly common in the study of herpesviruses, specifically in defining viral protein function. Many herpesvirus proteins bear little or no homology to cellular proteins, limiting the ability to infer functional roles based on amino acid sequence conservation. However defining the interacting partners for viral proteins in the context of infection is an effective means for identifying potential functional roles. Typically this approach involves generating a virus strain in which the protein of interest is fused to an epitope tag. The “tagged” viral protein is then affinity purified from infected cells lysates, and the associated host and/or viral proteins are identified by mass spectrometry (30). This approach has been used successfully to define novel functions for numerous herpesvirus proteins (e.g. (31–33), although it has yet to be extended to the study of roseolovirus protein functions.

A related approach can be used to identify changes in post-translation modifications (PTMs) of host and viral proteins during infection. Antibodies to a specific PTM are used as an affinity reagent, and the resulting immune complexes are analyzed by mass spectrometry. This approach is especially useful in defining changes in signaling pathways caused by infection. For example, phospho-proteomic analysis of MuHV4 infected cells identified virus-induced changes to multiple cellular signaling pathways (34), several of which are important for efficient virus replication. These approaches are easily extendable to the study of HHV6A, as a genetically tractable BAC clone exists as well as an efficient *in vitro* lytic replication model.

Quantitative whole cell proteomics (qWCP) is another approach used to identify changes in host protein expression during viral infection. qWCP approaches most commonly use a mass spectrometer to identify either relative or absolute quantities of proteins in cell lysates under different conditions. Both relative and absolute qWCP approaches are standardized, and we

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direct the reader to several method and review articles for additional details (35, 36). A modification of qWCP has also been developed that defines changes in cell surface proteins during infection (37). More recently whole cell proteomics coupled with transcript analysis has been used to identify and quantify the temporal expression of known and novel viral proteins (38). These studies have revealed that herpesvirus proteomes are far more complex than previously appreciated. Given the impact of proteomics on our understanding of herpesvirus protein function and genome complexity, applying these approaches to the study of roseoloviruses should rapidly increase our understanding of these complicated pathogens.

Metabolomics

Another –omics approach applied to the study of herpesvirus biology is metabolomics. The goal of metabolomics is to measure the abundance of all metabolites in a cell. Viruses are obligate intercellular pathogens that are directly reliant on host cell metabolites for anabolic processes; therefore herpesviruses must manipulate metabolic processes to support virus replication. Most metabolomics approaches utilize mass spectrometry to quantify a large number of metabolites from a single sample (reviewed in (39). Comparing the abundance of specific metabolites in virus-infected cells to that of mock-infected cells reveals critical virus-induced metabolic changes. Alternatively virus-induced changes to the rate of metabolism can be quantified by measuring the conversion of isotopically-labeled precursor metabolites such as glucose or glutamine into downstream metabolites.

Both approaches have been used to characterize how herpesvirus infection modulates metabolism. For example, both HHV-8 and HCMV increase aerobic glycolysis and stimulate fatty acid synthesis, reminiscent of the metabolic changes observed during oncogenesis (40–42). In contrast, HSV1 preferentially increases glucose metabolism by the pentose phosphate pathway, presumably to generate sufficient nucleotides for viral DNA replication (43). Fatty acid synthesis inhibitors limit HCMV replication (44) and the growth of primary effusion lymphomas associated with HHV-8 infection (45), and inhibitors of nucleotide metabolism decrease HSV1 replication (46). Therefore it is proposed that virus-induced changes in metabolism are promising targets for new antiviral therapeutics (47).

Based on the critical role for metabolic remodeling in the lytic replication cycle of other herpesviruses, we hypothesize that roseoleviruses modulate host metabolic pathways in similar yet distinct ways to support efficient virus replication. Uncovering mechanisms of virus-induced metabolic remodeling will likely result in new targeted therapeutic interventions directed at roseoloviruses. Unfortunately almost nothing is known of the effects of roseolovirus infection on host cell metabolism. As such, current drugs used to treat roseolovirus infections are based solely on inferred functions putatively shared with other herpesviruses (48). While defining new viral functions to target with novel antivirals will likely require much effort, virus-induced changes in metabolism should be relatively straightforward to define. Therefore metabolomics analyses of roseolovirus infections present a promising direction in the identification of new therapeutics to limit roseolovirus pathogenesis.

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Unmet Needs

While we have described different –omics applications that are accessible to roseolovirus researchers, we, as a field, lack several resources needed to advance our understanding of roseolovirus biology to the level of its herpesvirus cousins. For HHV-6B and HHV-7, the lack of an infectious BAC clone is perhaps the most significant barrier to –omics approaches. The absence of HHV-6B and HHV-7 BAC clones severely limits our ability to perform both forward and reverse genetic screens to define the complement of viral factors important for roseolovirus disease. The BACs would also provide a convenient starting point to generate a library of expression vectors for roseolovirus ORFs. This library would be a high value resource for forward genetics screens, such as screens to identify viral proteins that regulate the innate and adaptive immune response. This is arguably the most significant roadblock to roseolovirus research.

We also lack a sufficient understanding of genetic diversity amongst roseoloviruses. A functional genomics analysis of circulating roseolovirus strains in distinct patient populations or locations would likely prove invaluable for identifying viral pathogenesis determinants. In addition such an analysis would facilitate the development of roseolovirus diagnostics. Current PCR-based diagnostics for the assessment of viral load in patient samples vary greatly in sensitivity between laboratories (49). This may reflect differences in the primers used for detection, methods of nucleic acid isolation and/or the choice of standardization protocols. However strain variability across different geographical regions could also account for these discrepancies. A thorough genomics analysis of strain variation for each roseolovirus would provide the starting point for the development of diagnostics targeting invariant regions of roseolovirus genomes. These data would also allow for the generation of suitable reference strains that are needed to standardize diagnostic assays.

The ability to efficiently map viral transcription in a tissue or viral lifecycle specific manner is critical to understanding how these viruses grow and cause disease. Due to the reproducibility, low cost, high sensitivity and high specificity of tiled arrays (50), development of virus-specific arrays for HHV-6A -6B and -7 should be prioritized. The production and use of tiled arrays is a standard commodity at most institutions and offers a unified platform for transcript characterization and quantification. In addition RNA-Seq and other next generation sequencing approaches can identify novel coding regions (51), small RNAs (52, 53) and/or splice junctions (54) in herpesvirus genomes that may expand the roseolovirus proteome. A combinatorial transcriptomic approach is a priority for defining the coding capacity and regulatory regions of roseolovirus genomes.

As with functional genomics, the ability to perform directed proteomics experiments to define the function of HHV-6B and HHV-7 proteins is severely hampered by the lack of infectious BAC clones. However such approaches should be easily applicable to the study of HHV-6A, as the necessary genetic system exists and the required reagents are commercially available. In addition, mass spectrometry core facilities capable of producing and analyzing the data from proteomics experiments are common at most research institutions. As with transcriptomics, a combination of proteomics approaches is needed to define novel viral coding determinants and their functions in order to identify new targets for antiviral drugs.

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While metabolomics is less common than other –omics approaches in the study of herpesviruses, metabolomics has already identified virus-induced metabolic changes that can be targeted with drugs. Metabolic perturbations underlie several disease states, and the development of drugs that regulate metabolism is an active area of clinical research. In some cases, metabolomics studies have suggested that currently approved drugs have been found to have novel antiviral activity (47), potentially speeding translation of these findings into the clinic. Metabolomics approaches should be relatively straightforward and thus should be prioritized.

The rapid development of –omics approaches over the last ten years has greatly changed our view of herpesvirus biology. As described above, many standard –omics approaches can be easily adapted for roseolovirus research, although for studies of HHV-6B and -7 some hurdles still remain. The lack of -omics approaches to date in roseolovirus research as a whole presents an opportunity for researchers to coordinate an integrated and highly targeted –omics analysis of these clinically important human pathogens.

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Highlights

- High throughput protocols ("-omics") have uncovered mechanisms of herpesvirus pathogenesis.
- Although increasingly common, "-omics" assessment of roseoloviruses has yet to be performed.
- Reagents exist to study HHV-6A but resources need to be developed to study HHV-6B and HHV-7.

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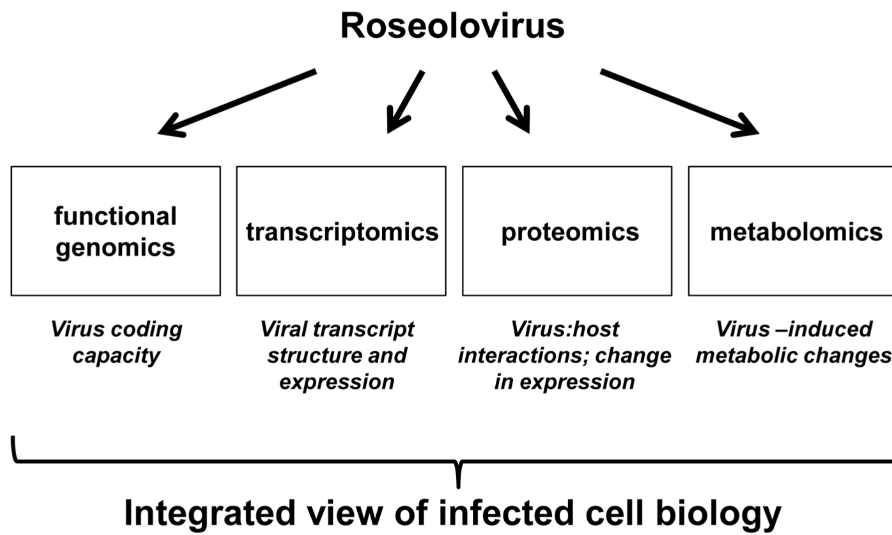

Figure 1. Schematic representation of high throughput system-wide analyses of roseolovirus pathogenic potential

The above is a listing of several available “-omics” analyses that are suited for profiling the impact of roseolovirus infection on host cells.