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Review

Virus hunting

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ABSTRACT

Viral diagnosis and discovery are receiving increasing emphasis with the recognition of their importance in addressing the challenges of emerging infectious and chronic diseases, and the advent of antiviral drugs with which to reduce the morbidity and mortality of viral infections. Here we review the status of the field including the use of molecular, proteomic and immunological assays for viral detection, social media platforms for surveillance, and public health investments that may enable enhanced situational awareness and insights into the origins of zoonotic viral diseases.

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Introduction

Two quotes from 1960 Nobel Laureate Peter Medawar are particularly apt in introducing this paper on virus hunting: "A virus is piece of bad news wrapped in protein" and "The human mind treats a new idea the same way the body treats a strange protein; it rejects it." The first is more often cited by clinicians and virologists; however, the latter has proven to be more reliable. Although viruses were once detected only in association with tissue pathology, the advent of molecular methods, and specifically high-throughput sequencing, has enabled the discovery of useful viruses. The retroviral envelope gene syncitin, for example, is critical to placental morphogenesis and may have contributed to mammalian evolution (Mi et al., 2000). Marine phages continuously lyse prokaryotes thereby releasing cellular components that are essential to the growth of phytoplankton, regulation of the global carbon cycle and may play roles in global climate change (Suttle, 2007; Danovaro et al., 2011). Viruses and viral gene products have also been harnessed in biotechnology and medicine. In this paper we will focus on viral discovery, diagnostics and surveillance in acute diseases; nonetheless, we ask the reader, in the spirit of Medawar's second quote, to consider the currently controversial concepts that viruses contribute to chronic disease as

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well as the potential role of viruses as tools for synthetic biology and medicine.

Diagnostics

Viral diagnosis was not a prominent service function in clinical microbiology laboratories until recently. This reflected resource intensive aspects of viral diagnostics such as culture using several cell lines, electron microscopy or serology as well as the fact that results provided insights with minimal impact of clinical management. The status of viral diagnostics has changed with the introduction of antiviral drugs and the development of molecular assays that can identify which patients will benefit from specific antiviral drugs, as well as monitor the adequacy of the responses to those drugs. Additionally, as demonstrated during the course of outbreaks of SARS in 2003, MERS in 2012 and Ebola in 2014, public health practitioners and policy makers are increasingly reliant on viral diagnostics to track emerging viral diseases and make decisions concerning who to isolate and for how long to do so.

Despite the shift in diagnostics toward molecular assays, viral culture continues to play an important role in virology because it is essential to test drugs, the neutralization capacity of antibodies and vaccine responses as well as to develop stocks of virus for work in animal models. Some viruses can be propagated in immortalized cell lines whereas others can only be grown in primary or organotypical cultures. Still others require the use of antibodies or RNAi to suppress innate immune responses or must be inoculated into live animals such as suckling mice. Gastrointestinal samples can be particularly challenging in that they typically contain more than one virus. We have seen many examples where a virus better adapted to culture may outgrow a virus that is more abundant in vivo, obscuring detection of the latter. Accordingly, we prefer to use less biased molecular methods for discovery and reserve culture for follow on studies.

Molecular assays

Molecular assays employed in clinical microbiology include polymerase chain reaction (PCR), isothermal amplification, DNA microarrays, in situ hybridization and sequencing. The most common are real time PCR assays wherein the release of a fluorescent molecule during the course of DNA strand replication results in detection of a single viral target. These assays are exquisitely sensitive, specific, quantitative and inexpensive, and are used not only for differential diagnosis but also to follow response to antiviral therapy. Portable PCR systems have been developed for field applications; nonetheless, some investigators prefer isothermal amplification tests that do not require programmable thermal cyclers. PCR sensitivity is highest when primers and probe sequences perfectly match the selected single genetic target. Indeed, these assays may fail to detect related viruses—a potentially daunting challenge in RNA virus infections where high mutation rates are characteristic. Consensus PCR assays wherein primers and/or probes contain wobble codes may succeed; however, they are typically less sensitive than specific PCR assays. Nested PCR tests that can employ consensus or specific primers in two sequential amplification reactions with either one (hemi-nested) or two (fully nested) primers located 3′ with respect to the first primer set may accommodate sequence variation and be as sensitive as fluorescent real time PCR assays. However, whereas in real time assays reporter readings are taken indirectly without opening the reaction vessels, nested PCR systems are prone to contamination because of the transfer of amplified product from the first to the second nested reaction.

Multiplex PCR assays are increasing in popularity because they can be used to simultaneously address a wide range of candidate viral, bacterial, fungal and parasitic pathogens. This is particularly important early in the course of infection when signs and symptoms of disease are less specific. However, multiplex assays can be difficult to establish because different primers and probes require different reaction conditions for optimal performance. An additional challenge is the limited repertoire of five fluorescent reporter molecules available for use in the most commonly employed clinical instruments. One alternative platform (Luminex xTag) employs flow cytometry to detect multiple PCR amplification products bound to matching oligonucleotides that are attached to fluorescent beads. By combining multiplex PCR amplification systems with various protocols for direct or indirect (tag-mediated) bead hybridization of the products, assay panels have been developed that detect more than 20 different genetic targets. Other PCR platforms employ mass spectroscopy to differentiate genetic targets based on product mass (IRIDICA, Abbott) or the presence of tags conjugated to primers used in PCR that vary in mass (Briese et al., 2005). Such systems can detect up to 20 different genetic targets in a single reaction but are typically 10–100 fold less sensitive than real time PCR.

Microarrays comprising millions of discrete oligonucleotide probes have the potential to detect all known viruses as well as viruses with limited homology to known viruses (Wang et al., 2002; Palacios et al., 2007); however, as they currently depend on a random (vs. specific) PCR amplification step followed by hybridization of the fluorescently labeled product, microarrays are even less sensitive than multiplex PCR systems. New platforms are in development that will detect viral sequence binding through changes in electrical conductance. These platforms will not require fluorescent scanners and may have improved sensitivity.

In situ hybridization is a method whereby nucleic acid probes are used to detect the presence and cellular distribution of complementary sequences in cultured cells or tissue sections. Its primary applications are for work focused on pathogenesis or cell biology rather than diagnostics.

High-throughput sequencing, also known as Next Generation Sequencing, has transformed medicine and virology by enabling viral discovery as well as diagnostics. Unlike PCR or array methods where the breadth of agents interrogated is limited by the capacity for multiplexing or known sequence data, high-throughput sequencing has the potential to simultaneously detect not only all viruses, but also bacteria, fungi and parasites. Furthermore, the time and resources previously required to clone and sequence entire viral genomes have been reduced from months to days. Over the past 10 years the cost has decreased 10,000 fold from $5000 per 1000 nucleotides in 2001 to $0.5 per 1000 nucleotides in 2012.

Platforms in current use analyze libraries of amplified nucleic acids. However, some platforms in development will have the capacity to directly sequence nucleic acid. Irrespective of the platform, raw sequence reads are filtered for quality and redundancy before assembly into contiguous strings of sequence streams that are aligned to sequences in databases using algorithms that search for similarity at the nucleotide and deduced amino acid levels in all six potential reading frames. The alignments allow identification of known and novel agents, as well as detection of genetic features that may be associated with drug or vaccine resistance, or provide insight into provenance and evolution.

Host response

Molecular diagnostic methods are sensitive, but do not serve in instances where microbial nucleic acids are not present in an accessible sample (e.g. the brain in encephalitis) or disease is triggered by an agent that is no longer present. Serology complements molecular diagnostics by providing insights into a subject's pathogen exposure history. It can test for the relevance of a discovery made using molecular methods by indicating that whether an

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