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Use of functional genomics to understand replication deficient poxvirus-host interactions

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ABSTRACT

High-throughput genomics technologies are currently being used to study a wide variety of viral infections, providing insight into which cellular genes and pathways are regulated after infection, and how these changes are related, or not, to efficient elimination of the pathogen. This article will focus on how gene expression studies of infections with non-replicative poxviruses currently used as vaccine vectors provide a global perspective of the molecular events associated with the viral infection in human cells. These high-throughput genomics approaches have the potential to lead to the identification of specific new properties of the viral vector or novel cellular targets that may aid in the development of more effective pox-derived vaccines and antivirals.

1. Introduction

The eradication of Smallpox, due to the elimination from nature of the causal agent of small-pox disease, Variola virus, was an important milestone in the history of medicine (Fenner, 1977, 1980, 1982). This goal was achieved after a global vaccination campaign using Vaccinia virus (VACV), a virus that is antigenically similar to Variola virus (Fenner, 1982; Smith, 2013). The efficacy of poxvirus vaccination was due to their immunogenic properties, including the ability to induce long-term humoral and cell-mediated immunity (Crotty et al., 2003). However, the vaccination campaign for the elimination of smallpox also revealed a significant incidence of complications, after immunisation with wild-type VACV, that ranged in severity from benign to lethal, especially in those individuals with reduced immune function (Bray, 2003). Thus, much subsequent research on VACV has focused on producing modified vaccines with improved safety profiles (Moss, 2013). Two main types of approaches have been taken to enhance the safety of VACV (Esteban, 2009; Garcia-Arriaza and Esteban, 2014; Sanchez-Sampedro et al., 2015). A first, more empirical strategy, consists of successive passage of the virus in an unnatural host or in tissue culture, and the isolation of virus variants (Gomez et al., 2009). The alternative approach, based on our increasing knowledge of the biology of the virus, includes the deletion of specific viral genes involved in important host interactions functions (Gomez et al., 2012b) (Paoletti et al., 1995). Both approaches have led to safe VACV strains.

Modified Vaccinia virus Ankara (MVA), derived from the parental Chorioallantois Vaccinia virus Ankara (CVA) by more than 570 passages in chicken embryo fibroblast (CEFs) cells, is now considered an attractive and promising candidate viral vector for the expression of foreign genes of interest because its unique properties (Antoine et al., 1998; Gomez et al., 2013; Meyer et al., 1991; Sutter and Staib, 2003). In particular, MVA, due to its avirulence and inability to replicate productively after *in vivo* inoculation, has a better safety profile than replication competent VACV, with similar levels of gene expression and better immunostimulatory properties (Antoine et al., 1998; Gomez et al., 2013; Meyer et al., 1991; Verheust et al., 2012). These characteristics have stimulated the development of MVA-based vaccines for a wide range of pathogens including malaria, leishmania (Moss, 1996; Schneider et al., 1998) and HIV, as well as for the treatment of cancer acting as a vector for delivery of effector molecules against tumors (Cebere et al., 2006; Corona Gutierrez et al., 2004; Gilbert et al., 2006; Harrop et al., 2006; Smith, 2013). MVA is also a potentially safe vaccine candidate for smallpox, in the hypothetical case of a recurrence of the virus as a bioterrorist weapon (Belyakov et al., 2003; Drexler et al., 2003; Earl et al., 2004; Wyatt et al., 2004).

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Another virus generated by serial passages in CEFs is ALVAC, a plaque-purified clone derived from an attenuated canarypox virus (Plotkin et al., 1995). ALVAC differs significantly from MVA in terms of genome size (approximately 365 kbp *versus* approximately 178 kbp, respectively) and in the number of open reading frames (ORFs) (McFadden, 2005). Many of ALVAC's ORFs may not be functional in mammalian cells, as evidenced by the inability of avipoxviruses to replicate in mammalian cells, and this characteristic may underlie its improved safety profile as a vaccine vector (Meyer et al., 1991; Taylor et al., 1988). ALVAC has been extensively evaluated in preclinical studies with both humans and non-human primates and widely used in human in a phase III clinical trial as an HIV/AIDS vaccine candidate in Thailand (Rerks-Ngarm et al., 2009).

The generation of attenuated viruses by the deletion of specific viral genes is based on the observation that many poxvirus genes are dispensable for growth*in vitro*. For VACV this approach has involved the deletion of viral genes that modulate the immune response, host-range and metabolism and has been extended to include the deletion of genes essential for viral replication by the use of complementing cell lines expressing the targeted VACV gene. The deletion of single immunomodulatory VACV genes from different strains has frequently led to attenuation of the virus as demonstrated in mice, sometimes accompanied by an increase in the immunogenicity of VACV antigens, as has been described for the VACV genes *E3L*(Beattie et al., 1996) (Jentarra et al., 2008), *B15R*/*B16R* (Staib et al., 2005), *A41L* (Clark et al., 2006), *B13R* and *A35R*(Rehm and Roper, 2011) or *C6L* (Sumner et al., 2013).

However the deletion of multiple viral genes can further enhance the attenuation and improve the safety of VACV as has been demonstrated by the engineering of the NYVAC strain of VACV (Brockmeier et al., 1993; Konishi et al., 1992). NYVAC is an attenuated derivative of the VACV strain Copenhagen (CopV), produced by the specific deletion of 18 ORFs, involved in host range, virulence and pathogenesis, from the genome of the parental virus (Tartaglia et al., 1992). Despite its limited ability to replicate productively in human and most mammalian cells, NYVAC provides a high level of foreign gene expression and efficiently triggers specific immune responses to these antigens in both experimental animals and humans. NYVAC-derived vectors are able to express antigens from a broad range of species (Tartaglia et al., 1992) and have been used as recombinant vaccines against numerous pathogens and tumors (Franchini et al., 2004; Kanesa-thasan et al., 2000; Myagkikh et al., 1996; Sivanandham et al., 1998) and it has shown strong and specific immunogenicity and a good safety profile in Phase I/II clinical trials against HIV-1 (Ockenhouse et al., 1998).

The attenuated MVA, NYVAC and ALVAC strains of poxvirus have become attractive vaccine vectors against HIV/AIDS. The arguments in favour of the use of these viruses as vaccine vectors include excellent immunogenicity and safety profiles and limited pre-existing immunity to poxvirus in the population at risk of HIV infection due to the abandonment of vaccine campaigns after the eradication of smallpox in the 1970s.The global HIV pandemic is not yet under control despite the reported recent decline in incidence (UNAIDS, 2013). According to the UNAIDS report for the year 2014, more than 35 million people live with HIV in world, with 2.1 million new infections each year, so that 69% of all people from sub-Saharan Africa live with HIV leading to 1.5 million deaths that can be attributed to HIV annually (UNAIDS, 2014). The effectiveness of the currently available HIV preventive and control interventions depends on strict adherence to a complex protocol (Abdool Karim et al., 2010; Cicconi et al., 2013; Vermund et al., 2013) with a threat of recidivism (Krcek, 1974). The search for an HIV vaccine during the past 25 years has been a challenge due to viral diversity and the ability of cells persistently infected to evade the immune system (Saunders et al., 2012). However, pre-clinical studies have identified immune and genetic biomarkers associated with protection against challenge that provide further insights for an HIV preventive vaccine for humans (Barouch et al., 2012). So far, there have been more than 180 clinical HIV-1 vaccine trials conducted in humans ranging from phase I to phase III (Garcia-Arriaza and Esteban, 2014; O'Connell et al., 2012; Vermund et al., 2013), including the recently concluded RV 144 phase III trial in Thailand using canarypox that showed a modest efficacy of 31% (Rerks-Ngarm et al., 2009). Given the biomedical importance of achieving a vaccine against HIV, a section of this review will focus on studies using genomic poxvirus vectors as vaccine against HIV.

The increasing use of high-throughput technologies is leading to an ever-more detailed view of virus-host interactions (Law et al., 2013; Peng et al., 2009; Tan et al., 2007; Tree et al., 2014). However, as the almost overwhelming amount of information from these "omics" experiments present in the databases grows, the choice of which elements of these vast amounts of data to investigate further becomes more difficult, but more important. In this regard, several examples of detailed studies of the biological relevance of a selected gene regulation after infection performed as follow-ups to microarray experiments have been carried out (Guerra et al., 2005, 2008) (Caceres et al., 2013). The aim of this article is to illustrate how data obtained from global gene expression experiments can be used to gain new insights into virus-host interactions, with particular emphasis on the immune response and its modulation by non-replicative poxvirus infection. The successful integration of this kind of information into attempts to understand biological processes at a systems level will be critical to the development of new vaccine vectors.

2. Overview of comparative genomic experiments performed with attenuated non-replicating poxvirus

2.1. Host gene regulation after MVA infection analysed by comparative genomic analysis

Many aspects of the biology of MVA remain only poorly understood, and a deeper understanding of the genetic factors that influence poxviral replication and viral gene function is needed to permit further optimization of the safety and immunogenicity of MVA derived vectors. It seems reasonable to suggest that comparative genomic experiments will be an important source of increased knowledge of the impact of MVA infection on human cells (Guerra et al., 2004, 2007; Royo et al., 2014).

The first experiments of comparative genomics using MVA virus were performed in HeLa cells, a cell line used in many key poxvirus biology studies because its high susceptibility to infection (Guerra et al., 2004). Cells were infected at 5 plaque-forming units (PFU) per cell to guarantee synchronous infection of all cells (Guerra et al., 2004), and the relative abundance of specific mRNAs were compared in MVA-infected cells and mock-infected cells at 2, 6 and 16 h postinfection (hpi). Compared to previous experiments using the same approach, but with the WR strain of vaccinia (Guerra et al., 2003), MVA infection was found to modulate a large number of immunomodulatory genes. Specifically, the transcription of several genes involved in the immune response (around 10 transcripts) was activated by MVA infection; six cytokines, interleukin*(IL) 1A* (I*L-1A), IL-6, IL-7, IL-8,* and*IL-15* and five members of the tumor necrosis factor receptor superfamily TNFRS, *TNFRSF7*,*TNFAIP3, TNFRSF14, TNFRSF17*, and *the TNF* receptor associated factor (TRAF) 3 (*TRAF3*) (Guerra et al., 2004). These data indicated that, in contrast to WR infection, MVA infection was associated with a more pronounced antiviral immune response of the host cell rather than a process beneficial for viral replication.

A clear upregulation of nuclear factor-κB (N*F-*κB*)* gene expression (mRNA and protein) was also a feature of MVA infection in