Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1784 (2008) 228–237
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbapap

Review

The role of cell signaling in poxvirus tropism: The case of the M-T5 host range protein of myxoma virus

Steven J. Werden, Grant McFadden *

Department of Molecular Genetics and Microbiology, College of Medicine, University of Florida 1600 SW Archer Rd., ARB Rm R5-295, PO Box 100332, Gainesville, FL 32610, USA

Received 20 June 2007; received in revised form 30 July 2007; accepted 2 August 2007
Available online 14 August 2007

Abstract

Poxviruses demonstrate strict species specificity in vivo that range from narrow to broad, however the fundamental factors that mediate the basis of poxvirus tropism remain poorly understood. It is generally believed that most, if not all, poxviruses can efficiently bind and enter a wide range of mammalian cells and all of the known host anti-viral pathways that block viral replication in nonpremissive cells operate downstream of virus entry. A productive poxvirus infection is heavily dependent upon the production of a vast array of host modulatory products that specifically target and manipulate both extracellular immune response pathways of the host, as well as intracellular signal transduction pathways of the individually infected cells. The unique pathogenesis and host tropism of specific poxviruses can be attributed to the broad diversity of host modulatory proteins they express. Myxoma virus (MV) is a rabbit-specific poxviruses that encodes multiple host range factors, including an ankyrin-repeat protein M-T5, which functions to regulate tropism of MV for rabbit lymphocytes and some human cancer cells. At the molecular level, M-T5 binds and alters at least two distinct cellular proteins: Akt and cullin-1. The direct interaction between M-T5 and Akt was shown to be a key restriction determinant for MV tropism in a spectrum of human cancer cells making MV an excellent oncolytic candidate. Thus, the intricate relationship between viral encoded proteins and components of the host cell signaling networks can have profound impact on poxvirus tropism. The lessons we continue to learn from poxvirus host range factors like M-T5 will provide further insights into the factors that regulate poxvirus tropism and the mechanisms by which poxviruses micromanipulate the signaling pathways of the infected cell.

Keywords: Poxvirus; Virus tropism; Akt; Ankyrin repeat; F-box; Protein kinase

1. Introduction

Poxviruses are complex viruses characterized by a large linear double-stranded DNA genome, a large, enveloped brick-shaped virion, and replication exclusive to the cytoplasm [1]. Our lab has extensively studied one poxvirus, myxoma virus (MV), as a model of how poxviruses evade the immune system and micromanage the anti-viral responses of the infected host. MV is a natural pathogen in rabbits (*Sylvilagus* sp.) of the Americas, resulting in a benign infection, and characterized by a cutaneous fibroma restricted to the site of inoculation [2]. However, when the virus infects European rabbits (*Oryctolagus cuniculus*), MV causes myxomatosis, a rapid systemic and highly lethal infection [3]. The pathogen–host relationship between MV and the European rabbit is well characterized [4,5] and provides an excellent model to study the mechanism by which large DNA viruses manipulate the host immune responses during virus infection of an immunocompetent host. Successful MV replication and dissemination can be attributed to the ability of the virus to avoid recognition and clearance by the host innate and acquired immune system in the infected rabbit [6]. Similar to other sequenced poxviruses, the MV genome contains a set of highly conserved poxvirus genes centrally located in the viral genome

Abbreviations: ANK, ankyrin; BGMK, baby green monkey kidney; CHO, Chinese hamster ovary; INF, interferon; IRF-3, interferon regulatory factor 3; MNF, myxoma nuclear factor; mTORC, mammalian target of rapamycin complex; MV, myxoma virus; PDK1, 3-phosphoinositide-dependent protein kinase-1; PH, pleckstrin homology; PI-3K, phosphatidylinositol-3-kinase; PIKE-A, phosphatidylinositol-3-kinase enhancer Akt; pMEF, primary mouse embryo fibroblast; RK13, rabbit kidney fibroblast; SCF, Skp, Cullin, F-box; Skp, S-phase kinase-associated protein; STAT1, signal transducer and activator of transcription 1; VARV, variola virus; VSV, vesicular stomatitis virus; VV, vaccinia virus

* Corresponding author. Tel.: +1 352 273 6852; fax: +1 352 273 6849.
E-mail address: grantmcf@ufl.edu (G. McFadden).

1570-9639/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbapap.2007.08.001

[7]. These particular conserved genes primarily encode proteins necessary for viral replication and formation of progeny virions. In contrast, genes located towards the ends of the genome, either within the terminal inverted repeats or near-terminal regions, encode proteins with greater diversity among the poxvirus family and are critical for immunomodulatory properties and host range functions of the individual poxvirus member [6].

Despite considerable advances in the understanding of poxvirus replication, the fundamental mechanisms that mediate the basis for host range and poxvirus tropism remains poorly understood [8]. Recent data suggest successful poxvirus replication in any particular host species or cell type is dependent upon the production of a vast array of host modulatory products that specifically target and manipulate both extracellular immune response pathways as well as intracellular signal transduction pathways [6]. Such sophisticated strategies employed by poxviruses enable them to establish an environment within permissive infected cells that favors virus replication, whereas nonpermissive cells are able to abort the viral replication cycle, usually at a point downstream of virion binding and entry. In doing so, most poxviruses have the capacity to initiate infection of a broad spectrum of vertebrate cells in vitro, whereas individual poxvirus family members usually exhibit strict host species specificities in vivo. The unique pathogenesis and host tropism of individual poxviruses can be attributed to the broad diversity of host immunomodulatory proteins they express. In fact, no single immunomodulatory ortholog that is common to all poxviruses has been identified at this point in time [9].

Like all poxviruses, MV expresses a distinct repertoire of virulence factors that have been experimentally shown to subvert the host immune and anti-viral responses to virus infection and mediate MV pathogenicity [10]. Included among these viral encoded proteins are homologs of cellular cytokine receptors (viroreceptors), secreted mimics of host ligands or regulators (virokines) [11] and inhibitors of the apoptotic pathway [12,13]. In the absence of these immunomodulatory factors, for example in MV constructs in which individual viral genes have been deleted, the myxomatosis disease progression in susceptible rabbits is often considerably attenuated, thus demonstrating their importance in mediating the ability of MV to evade the host immune system and establish a successful infection. In general, MV proteins that affect viral pathogenesis or dissemination are termed virulence factors, and those which specifically mediate the tropism of MV in specific cell types or tissues are referred to as host range factors. Most of the known MV host range factors are intracellular proteins that manipulate the intracellular environment of the infected cell.

2. M-T5, a MV encoded host-range gene

Host range factors are a specific class of poxvirus-encoded modulatory proteins that have been implicated in the determination of host range at either the cellular or tissue level. Each poxvirus possesses its own specific set of host range genes that confer the tropism phenotype that is unique to that virus. These host range genes express proteins that employ various intracellular strategies to evade diverse antiviral pathways and mediate viral tropism at some level. A variety of poxvirus host range genes have been characterized to date, but usually these viral genes were discovered to confer host range properties only when a gene knockout virus has been constructed and then tested for the ability to replicate in the same spectrum of cells and tissues for which the parental virus is known to be permissive.

Of the known poxvirus host range genes described to date, the MV M-T5 gene was first discovered over a decade ago to be critical for MV replication within rabbit T-lymphocytes [14]. M-T5 possesses no extensive sequence similarity to non-viral proteins but does share some sequence similarity with vaccinia virus (VV) gene B4R and the variola virus (VARV) B6R. Currently, although little is known about the function of these particular gene products, similarity between the ankyrin (ANK) repeats of M-T5 and the well-studied host-range gene CHOhr has been previously noted [15]. The cowpox virus CHOhr gene encodes a 77-kDa protein that was initially identified for its ability to rescue VV replication in normally nonpermissive Chinese hamster ovary (CHO) cells [16–19]. VV also possesses two additional host range genes, named K1L and C7L, which have been demonstrated to be required for productive virus growth in RK13 and HeLa cells, respectively [20–22]. Viral replication of VV deficient in either or both C7L and K1L can be rescued in restrictive cells by the expression of the CHOhr gene product [21]. Additionally, the CHOhr gene was shown to functionally replace K1L and permit VV replication in RK13 cells, suggesting that expression of CHOhr also possesses rabbit cell host range properties [18]. It is relevant to note that when M-T5 is compared to CHOhr, sequence similarity between the two extends across the entire length of the protein with the exception of a 106 amino acid deletion in the central region of M-T5. The functional significance of this apparent internal deletion remains yet to be determined [14].

Two copies of the M-T5 open reading frame (ORF) are present in the MV genome, one within each copy of the virus terminal inverted repeat (Fig. 1) [7]. The M-T5 gene is 1452 nucleotide in length and encodes a protein of 483 amino acids, which is expressed rapidly following infection and remains as an abundant and stable 49-kDa cell-associated protein throughout the course of viral infection [14,23]. To begin to understand the functional role of M-T5 during virus infection, a recombinant MV was constructed in which both copies of the M-T5 gene were disrupted by the insertion of a selectable marker, namely beta-galactosidase [14]. The replication kinetics of the M-T5 deficient MV (vMyxT5KO) in cultured rabbit kidney fibrobasts (RK13) was indistinguishable from cells infected by wild-type MV. However, rabbit T-lymphocytes infected with vMyxT5KO resulted in an abortive infection characterized by rapid inhibition of both viral and host gene synthesis accompanied by extensive cellular apoptosis. [14]. Thus, it would appear that M-T5 specifically promotes MV replication in lymphocytes by preventing the nonspecific shutdown of protein synthesis, which is probably the stimulus leading to the induction of apoptosis that aborts the infection of rabbit lymphocytes with the vMyxT5KO virus. Similarly, host range studies of VV demonstrated early and extensive inhibition of viral and host range protein synthesis