Virus Research 194 (2014) 191–199

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Review

Coronavirus non-structural protein 16: Evasion, attenuation, and possible treatments

Vineet D. Menachery[a,*], Kari Debbink[a,b], Ralph S. Baric[a,b]

[a] Department of Epidemiology, University of North Carolina, Chapel Hill, NC 27599, USA
[b] Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC 27599, USA

ARTICLE INFO

Article history:
Received 14 August 2014
Received in revised form
18 September 2014
Accepted 19 September 2014
Available online 30 September 2014

Keywords:
CoV
SARS-CoV
2′O-Methyl-transferase
2′O-MTase
NSP16
MDA5

ABSTRACT

The recent emergence of Middle East Respiratory Syndrome Coronavirus (MERS-CoV), nearly a decade after the Severe Acute Respiratory Syndrome (SARS) CoV, highlights the importance of understanding and developing therapeutic treatment for current and emergent CoVs. This manuscript explores the role of NSP16, a 2′O-methyl-transferase (2′O-MTase), in CoV infection and the host immune response. The review highlights conserved motifs, required interaction partners, as well as the attenuation of NSP16 mutants, and restoration of these mutants in specific immune knockouts. Importantly, the work also identifies a number of approaches to exploit this understanding for therapeutic treatment and the data clearly illustrate the importance of NSP16 2′O-MTase activity for CoV infection and pathogenesis.

Contents

1. Introduction

Coronaviruses represent important human pathogens that have emerged multiple times from zoonotic reservoirs over the past few centuries (Becker et al., 2008; Graham et al., 2013; Li et al., 2005; Nicholls et al., 2003; Perlman and Netland, 2009). In the aftermath of severe acute respiratory syndrome coronavirus (SARS-CoV) emergence in 2003, substantial efforts were made to improve our understanding of CoV infection and pathogenesis in order to develop novel therapeutics for current and future CoV mediated outbreaks (Perlman and Netland, 2009). Despite significant advances over the past decade, broadly effective treatments for SARS-CoV remain elusive. Coupled with the recent emergence of both Middle East Respiratory Syndrome

* Corresponding author at: Department of Microbiology and Immunology, 3304 Michael Hooker Research Building, Campus Box 7435, Chapel Hill, NC 27599-7435, United States. Tel.: +1 919 966 3895.
E-mail addresses: vineet@email.unc.edu, Vineet.Menachery@gmail.com (V.D. Menachery).

http://dx.doi.org/10.1016/j.virusres.2014.09.009
0168-1702/

(MERS) CoV in 2012 (Corman et al., 2012; Josset et al., 2013) and a virulent strain of porcine epidemic diarrhea virus (PEDV) (Huang et al., 2013), the lack of progress underscores the continued need to study and develop treatment for this family of viruses.

As the SARS-CoV outbreak progressed, computational models predicted a number of enzymatic functions conserved in the viral poly-protein including a conserved 2′O-methyl-transferase activity (2′O-MTase) for CoV non-structural protein 16 (NSP16) (Snijder et al., 2003). While unknown at the time, subsequent immunity research revealed a role for 2′O-methylation of viral mRNA in distinguishing between self/non-self (Daffis et al., 2010; Zust et al., 2011). The presence of these host and virus effectors suggested the importance of 2′O-methylation as a virulence determinant during CoV infection. In this review, we examine NSP16 interaction partners and activity as well as the factors that mediate host responses. In addition, the manuscript explores approaches to exploit these pathways for therapeutic treatment of both current and emergent disease. Overall, the review seeks to update the current state of the CoV NSP16 field and possibilities for future therapeutics based on targeting 2′O-MTase activity and corresponding immune responses.

2. mRNA capping and host recognition

Overcoming the host immune response is paramount to the success of any viral infection. Since the immune response is predicated on recognition, viruses have evolved means to disrupt host sensing through either direct antagonism of pathway components or molecular mimicry of host processes (Decroly et al., 2012). An important example of the latter is the duplication of capping elements for viral mRNAs (Decroly et al., 2012). Eukaryotic hosts utilize a 5′-terminal capping system to promote efficient nuclear export, robust translation, and enhanced stability of host mRNA. In addition, mRNA capping structure also helps to distinguish between self/non-self RNA and can lead to initiation of the host immune response. In recent years, unprotected 5′-triphosphates on nascent RNA (Fig. 1A) has been identified as part of the recognition trigger for host sensor molecule retinoic acid inducible gene I (RIG-I) leading to down stream type I IFN induction (Hornung et al., 2006; Myong et al., 2009). Similarly, both IFN-induced protein with tetratricopeptide repeats (IFIT) 1 and IFIT5, highly induced interferon-stimulated genes (ISGs), also bind exposed 5′-triphosphate on viral RNA, interfering with their activity through competition with EIF4E (Diamond, 2014; Pichlmair et al., 2011; Habjan et al., 2013). Triphosphate cleavage and addition of the 7-methylguanosine cap effectively protect host mRNA from this particular targeting (Fig. 1B–D). However, unmethylated 2′O on the ribose of cap-0 RNA has also been identified as a pathogen associated molecular pattern (PAMP); recognized by host sensor molecule Melanoma Differentiation-Associated protein 5 (MDA5) as well the effector IFIT family, the absence of 2′O-methylation on viral RNA induces a more robust type I IFN response that attenuates viral replication and infection *in vitro* and *in vivo* (Fig. 1E) (Daffis et al., 2010; Zust et al., 2011). In contrast, viral mRNA maintaining both the 7-methylguanosine cap and 2′O-methylation (caps 1 and 2, Fig. 1F) remains viable to levels similar to host mRNA. Together, recognition of these moieties and the subsequent host response indicate the importance of mRNA capping restrictions as a barrier to viral infection. These findings also raise the possibility that capping activities may preferentially function as a defense mechanism against cell intrinsic antiviral effectors, suggesting the possibility that viral mRNA are preferentially translated by cap-independent mechanisms.

3. Coronavirus capping

Naturally, viruses have evolved a variety of mechanisms to overcome these capping restrictions. For viruses that replicate within the nucleus including most DNA viruses and retroviruses, using the host capping machinery provides the primary means of protecting mRNA from restriction (Decroly et al., 2012). Similarly, other viral families including orthomyxoviruses, arenaviruses, and bunyaviruses, employ “cap snatching” to excise cap structures from early host RNAs and incorporated them into nascent viral RNAs. Finally, many viral families encode their own capping machinery that either mimics the sequential eukaryotic approach or an alternative approach to effectively maintain translation and block aspects of host immune recognition. Examples of the later approach include VpG-like viral proteins that mimic cap structures like picornaviruses and caliciviruses that bind 5′ viral RNA acting as a cap (Decroly et al., 2012); similarly, RNA secondary structure including internal ribosome entry sites (IRES) elements permit translation of viral RNA and may offer minor protection from host recognition (Hyde et al., 2014). Importantly, a significant portion of viruses encode their own viral capping proteins that directly copy the host machinery, making it indistinguishable from host mRNA and neutralizing an important barrier to viral infection.

For coronaviruses, a series of highly conserved non-structural proteins (NSPs) have been identified in capping viral RNA. Detailed reports have identified or predicted roles for NSP13 (Ivanov et al., 2004; Ivanov and Ziebuhr, 2004), NSP14 (Chen et al., 2009), and most recently, the NSP10/NSP16 complex (Bouvet et al., 2010; Chen et al., 2011; Debarnot et al., 2011; Decroly et al., 2008, 2011; Lugari et al., 2010) in capping of CoV RNA. Similar to the host process, the capping is believed to be initiated by a RNA phosphatase that removes a phosphate group from the nascent mRNA (Fig. 1A); for CoVs, this process has been attributed to the 5′–3′ helicase/NTPase NSP13, although not yet confirmed by experimental examination (Fig. 1B) (Snijder et al., 2003). Thereafter, an undetermined host or viral guanyly transferase (GTase) mediates cleavage of GTP to GMP and covalent attachment to the diphosphate linked mRNA (Fig. 1C). Next, NSP14, an SAM-dependent MTase, facilitates the addition of a methyl group to the guanosine at N7, producing a viral mRNA cap-0 structure (Fig. 1D). Following this step, the viral RNA is effectively protected from RIG-I recognition of the free 5′ triphosphate; however, absent 2′O-methylation, the viral mRNA will still trigger the sensor MDA5 and IFIT effectors (Fig. 1E). The mRNA cap for CoVs is completed by NSP16, an SAM-dependent nucleoside-2′O-methyl-transferase, that ensures formation of a protective cap-1 structure that prevent recognition by either MDA5 or IFIT proteins (Fig. 1F). Finally, the NSP16/NSP10 complex finishes CoV capping process permitting viral infection with reduced host recognition.

4. Structural conservation and catalytic tetrad

CoV 2′O-MTase belongs to the RrmJ/fibrillarin superfamily of ribose 2′O-methyl-transferases conserved in a number of host cellular homologues as well as viral orthologs in Flaviviruses, Alphaviruses, and Nidoviruses (Feder et al., 2003). This family of proteins catalyzes the transfer of S-adenosylmethionine (SAM) methyl group to methyl acceptors and relies on a conserved K-D-K-E tetrad within the substrate binding pocket for activity. Substitution at any of three residues (K-D-K) resulted in ablation of catalytic activity (Feder et al., 2003). In addition to the tetrad, the canonical members of this enzyme family including catechol O-MTase, maintain a conserved structural motif of seven-stranded B-sheets flanked by three alpha helices on each side that constitute both the acceptor substrate and SAM-binding domains (Martin