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Nidovirus papain-like proteases: Multifunctional enzymes with protease, deubiquitinating and deISGylating activities

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ABSTRACT

Coronaviruses and arteriviruses, members of the order *Nidovirales*, are positive strand RNA viruses that encode large replicase polyproteins that are processed by viral proteases to generate the nonstructural proteins which mediate viral RNA synthesis. The viral papain-like proteases (PLPs) are critical for processing the amino-terminal end of the replicase and are attractive targets for antiviral therapies. With the analysis of the papain-like protease of Severe Acute Respiratory Syndrome coronavirus (SARS-CoV), came the realization of the multifunctional nature of these enzymes. Structural and enzymatic studies revealed that SARS-CoV PLpro can act as both a protease to cleave peptide bonds and also as a deubiquitinating (DUB) enzyme to cleave the isopeptide bonds found in polyubiquitin chains. Furthermore, viral DUBs can also remove the protective effect of conjugated ubiquitin-like molecules such as interferon stimulated gene 15 (ISG15). Extension of these studies to other coronaviruses and arteriviruses led to the realization that viral protease/DUB activity is conserved in many family members. Overexpression studies revealed that viral protease/DUB activity can modulate or block activation of the innate immune response pathway. Importantly, mutations that alter DUB activity but not viral protease activity have been identified and arteriviruses expressing DUB mutants stimulated higher levels of acute inflammatory cytokines after infection. Further understanding of the multifunctional nature of the Nidovirus PLP/DUBs may facilitate vaccine development. Here, we review studies describing the PLPs' enzymatic activity and their role in virus pathogenesis.

1. Introduction

How do you pack a lot of information into a small space? This is the challenge for all microbes and analysis of viral genomes reveals interesting strategies of genetic economy. Positive strand RNA viruses employ genetic economy by encoding polyproteins that are processed by viral proteases during replication. This strategy minimizes genome size by allowing for the expression of multiple protein products from a single open reading frame. An additional twist to this genetic economy is that the viral protease itself may be multifunctional, *i.e.* the protease may act on both viral and host cell proteins. Hepatitis C virus encodes a polyprotein that is processed by a viral protease, NS3/4a, that also cleaves host cell mitochondrial associated viral sensor (MAVS), thus inactivating the innate immune response to viral infection (Li et al., 2005). Poliovirus 2A protease processes the viral polyprotein and host cell factor eIF4G, which shifts the ribosomes from cap-dependent to cap-independent translation, whereas 3C protease cleaves poly(A)-binding protein to facilitate the complete host translation shutoff (Kuyumcu-Martinez et al., 2004; Gradi et al., 1998). Here, we focus on coronaviruses and arteriviruses, two families of positive strand RNA viruses in the order *Nidovirales*, and review recent findings illuminating the mutifuctionality of the papain-like proteases (PLPs) encoded in the replicase polyproteins. For these viruses, PLPs play a critical role in processing the amino-terminal portion of the replicase polyprotein and are attractive targets for antiviral drug development. In addition, structural studies have revealed a striking similarity of the viral PLPs to cellular deubiquitinating enzymes (DUBs), which are involved in regulation of the innate immune response to viral infection. This raises the question of the multifunctional potential of Nidovirus PLPs and their role in antagonism of the innate immune response to viral replication. In this review we discuss current knowledge on the multifunctionality of PLPs encoded within coronaviruses and arteriviruses genomes and their potential role in viral pathogenesis (summarized in Table 1).

The *Coronaviridae* is a family of enveloped, positive strand RNA viruses with very large genomes ranging in size from 28 to 32 kilobases. Coronaviruses were identified as etiologic agents of

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Table 1
Coronavirus and arterivirus papain-like protease characteristics.

Virus family	Virus	Protease characteristics	Reference
Coronaviridae	*Betacoronavirus*		
	SARS-CoV PLpro	Proteolytic activity	Harcourt et al., J Virol. (2004)
		Crystal structure	Ratia et al., PNAS (2006)
		DUB activity *in vitro* toward K-48 Ub_2 and Ub_7, and K-63 Ub_7	Barretto et al., J Virol. (2005); Lindner et al., J Virol. (2005)
		DUB activity in cell culture	Frieman et al., J Virol (2009)
		Blocks IFNβ induction	Devaraj et al., J Biol Chem. (2007)
		DeISGylating activity *in vitro*	Lindner et al., ABB (2007)
	MERS-CoV PLpro	Proteolytic activity	Kilianski et al., J Virol. (2013)
		DUB and deISGylating activities in cell culture	Mielech et al., Virology (2014);
		Blocks IFNβ induction	Yang et al., JGV (2014)
	MHV PLP2	Proteolytic activity	Kanjanahaluethai and Baker, J Virol. (2000); Kanjanahaluethai et al., J Virol. (2003)
		DUB activity in cell culture	Zheng et al., Cell Res.
		Blocks IFNβ induction	(2008)
		Deubiquitinates TBK-1 and IRF3 in cell culture	Wang et al., PLoS ONE (2011)
	Alphacoronavirus		
	HCoV-NL63 PLP2	Proteolytic activity and DUB activity *in vitro* toward K-48 Ub_6	Chen et al., J Virol. (2007)
		DUB activity *in vitro* toward K-48 and K-63 Ub_6, and in cell culture	Clementz et al., J Virol.
		DeISGylating activity in cell culture	(2010)
		Blocks RIG-I mediated IFNβ	
	TGEV PLP1	Proteolytic activity	Wojdyla et al., J Virol.
		Crystal structure	(2010)
		DUB activity *in vitro* toward K-48 and K-63 polyubiquitin chains	
	PEDV PLP2	DUB activity in cell culture	Xing et al., J Gen Virol.
		Blocks IFNβ induction	(2013)
		Deubiquitinates RIG-I	
Arteriviridae	EAV PLP2	Proteolytic Activity	Snijder et al., J Biol Chem. (1995)
		DUB and deISGylating activity in cell culture	Frias-Staheli et al., CHM (2007)
		DUB activity *in vitro* toward K-48 and K-63 Ub_7 chains	van Kasteren et al., J
		Blocks IFNβ induction	Virol. (2012)
		DUB activity in cell culture; Deubiquitinates RIG-I	
		Crystal structure with ubiquitin	van Kasteren et al.,
		DUB activity is required for innate immune inhibition in infected cells	PNAS (2013)
	PRRSV PLP2	Proteolytic activity	Han et al., J Virol. (2009); Han et al., J Virol. (2010)
		DUB and deISGylating activity in cell culture	Frias-Staheli et al., CHM (2007)
		Blocks IFNβ induction	Sun et al., J Virol. (2010)
		DeISGylating activity in cell culture	Sun et al., J Virol. (2012)

respiratory, gastrointestinal and neurologic diseases in humans and other animals. Coronaviruses are now notorious for emerging from animal reservoirs into the human population, sometimes with pandemic potential. In 2002, the coronavirus responsible for the pandemic of Severe Acute Respiratory Syndrome (SARS) emerged from Chinese horseshoe bats, and through an intermediate host (likely civet cats), into humans. This resulted in over 8000 infections with 10% mortality (Perlman and Netland, 2009). The SARS pandemic was controlled by public health measures of isolating infected individuals and their contacts which disrupted the chain of human to human transmission. Since 2012, a coronavirus designated Middle East Respiratory Syndrome Coronavirus (MERS-CoV) has been detected in 178 patients with a reported mortality of 43% (www.who.int, 2014). MERS-CoV-like sequences have been detected in bats suggesting bats as a potential reservoir of the virus (Memish et al., 2013). In addition, camels have been implicated as a potential intermediate host since the discovery of MERS-CoV neutralizing antibodies and infectious virus in dromedary camels (Reusken et al., 2013; Haagmans et al., 2014; Meyer et al., 2014). Additional endemic human coronaviruses that cause respiratory tract disease include: HCoV-229E and HCoV-OC43, which cause common colds; HCoV-NL63, which has been associated with croup in children; and HCoV-HKU1, associated with lower respiratory tract infection and pneumonia in the elderly. Coronaviruses are also important pathogens of livestock and pets including transmissible gastroenteritis virus (TGEV) (pigs), porcine epidemic diarrhea virus (PEDV) (pigs); bovine coronavirus (cows); infectious bronchitis virus (chickens); feline coronavirus (cats); and canine coronavirus (dogs). Interestingly, we now recognize that bats harbor diverse strains of coronaviruses, and these bat viruses may be the ancestors of the "species specific" viruses (Lau et al., 2013). The identification of a common therapeutic target in the genomes of coronaviruses may allow for the development of a broad spectrum antiviral therapy to existing and potentially emerging coronaviruses.

2. Coronavirus papain-like proteases and their role in virus replication

All coronaviruses replicate in the cytoplasm of infected cells through the action of the viral replicase complex. The coronavirus replicase is produced upon translation of the incoming RNA genome. The replicase gene contains two open reading frames, ORF1a and ORF1b, which are connected by a frame shift region allowing for translation of the ORF1ab polyprotein. The replicase polyproteins, designated pp1a and pp1ab, are processed into 16 non-structural proteins (nsps) by two or three, depending on the coronavirus, viral proteases. The PLPs are responsible for the cleavage of the amino-terminal portion of the polyprotein. Coronavirus PLP activity was identified by *in vitro* transcription/translation studies of genomic RNA and the recognition that the polyprotein product was processed by an encoded protease domain (Denison and Perlman, 1986). Site-directed mutagenesis and deletion studies reveal that for the model coronavirus, mouse hepatitis virus (MHV), there are two PLP domains with PLP1 processing the polyprotein between nsp1/nsp2 and nsp2/nsp3 (Denison et al., 1992; Baker et al., 1989, 1993; Teng et al., 1999). Further

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