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Genetic deficiency and polymorphisms of cyclophilin A reveal its essential role for Human Coronavirus 229E replication

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Replication of coronaviruses is inhibited *in vitro* by cyclosporin A, a well-known immunosuppressive drug which binds to cellular cyclophilins thus inactivating their enzymatic cis-trans peptidyl-prolyl isomerase function. Latter is required for proper folding of cellular proteins and of proteins of several viruses. Here, we summarize present knowledge on the role of cyclophilin A during coronavirus replication. We present data on the effect of cyclophilin A single nucleotide polymorphism mutants on the replication of human CoV-229E demonstrating the requirement of proper cyclophilin A function for virus propagation. Results define cellular cyclophilin A as a host target for inhibition of coronaviruses ranging from relatively mild common cold to highly pathogenic SARS-CoV and MERS-CoV viruses with the perspective of disclosing non-immunosuppressive cyclosporin A analogs to broadly inactivate the coronavirus family.

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Introduction

Coronaviruses (CoVs) infect a variety of mammalian species including bats, mice, cats, birds and humans causing infection of respiratory and gastrointestinal tracts and the central nervous system [1]. CoVs are enveloped viruses containing the largest known single-stranded RNA genomes (25–32 kb) with positive-sense orientation. They are divided into four genera: *Alpha-* (HCoV-229E), *Beta-* (SARS-CoV: lineage B; MERS-CoV: lineage C), *Gamma-* and *Deltacoronavirus* [2]. The six human CoVs, namely HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV and MERS-CoV mainly target the respiratory tract. 15–30% of common colds are caused by HCoVs (229E, OC43, NL63, HKU1) with mostly seasonal occurrence. Whereas 229E and OC43 are known since the mid-1960s, SARS-CoV appeared first in China causing a worldwide outbreak with 8098 cases and 774 deaths in 2002/03 and with enormous socio-economic impact [3]. Arising interest in CoVs led to the discovery of NL63 in 2004 [4] and HKU1 in 2005 [5]. MERS was identified in 2012 in Saudi Arabia. By 31 May 2015 MERS-CoV infections rose to 1154 cases with 431 deaths [6]. In May 2015 a new outbreak occurred in South Korea with over 120 reported cases, 10 deaths as of 11 June [7], and over 2300 individuals placed under quarantine, making it the largest outbreak outside Saudi Arabia.

Until now no effective drug treatment is available neither against the common cold nor the highly pathogenic CoVs. Development of antivirals has concentrated on the development of protease [8,9] and helicase inhibitors [10,11]. Great efforts have been made to discover anti-MERS agents by screening defined drug libraries [12–14]. Although, CoVs display some proofreading activities during replication viral targets are usually prone to develop resistance mutations rather quickly. Therefore, defining cellular co-factors required for viral replication as targets is rather intriguing. Screening 16,671 diverse compounds for anti-229E activity Lundin *et al.* have identified an inhibitor (K22) specifically targeting membrane-bound coronaviral RNA synthesis at an early step of viral replication [15]. Using unbiased high-throughput protein–protein interaction screening methods we identified cyclophilins as binding factors for CoV proteins and its inhibitor cyclosporin A (CsA) as broad-range anti-coronaviral agent [16••]. CsA binding and inactivation of CypA as cellular co-factor for virus propagation is summarized in the accompanying article in the October issue of Current Opinion in Virology by von Hahn and Ciesek [17]. Here we summarize the inhibitory effect of CsA and non-immunosuppressive derivatives thereof on CypA function during CoV replication. We further describe the effect of the genetic deficiency and of individual CypA SNP mutants on the replication of HCoV-229E indicating the requirement of correctly folded enzymatic groove of CypA.

Cyclophilins and inhibitors

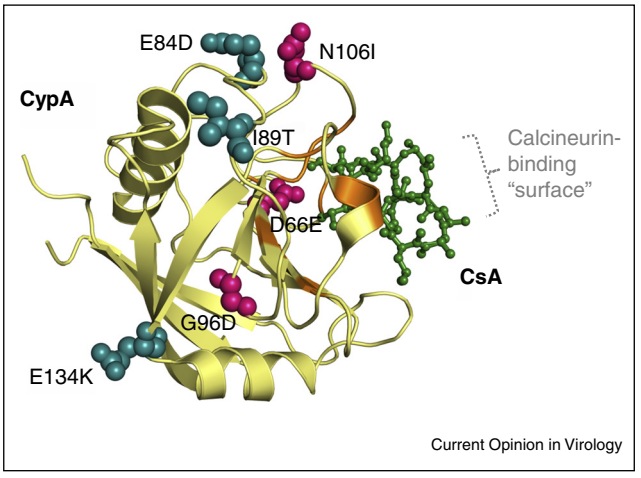
Cyclophilins and FKBPs are members of two ubiquitously distributed PPIase families, collectively called immunophilins [18••]. They are important for a number of cellular processes, for example, protein folding, maturation, trafficking, signal transduction, cell differentiation, apoptosis and infections. CypA and CypB were recognized in 1993 by Y2H techniques to specifically bind to the HIV-1 Gag polyproteins Pr55gag and to capsid p24, but only CypA was demonstrated to be specifically incorporated into HIV-1 virions [reviewed by 19••]. Binding of the amino-terminal domain of HIV-1 capsid to the active groove of CypA was demonstrated by crystal structure [20] and by mutational analysis [21,22]. Proline-containing peptide substrates bind to this hydrophobic pocket conferring them enzymatic peptidyl-prolyl *cis/trans* isomerase (PPIases, EC number 5.2.1.8) activity. The isomerase function of Cyp was identified already in 1989 [23•]. It was first shown for HIV-1 that CsA also binds to the groove thus interfering with proper capsid formation and virus replication. In the case of Hepatitis C Virus (HCV) the involvement of Cyps was shown by several groups. Initially conflicting results on which CypA or B was supporting HCV replication could be clarified in favor of CypA [24–26].

A very important, but from PPIase activity completely independent feature of CsA binding to CypA is the formation of a tri-molecular complex with the cellular phosphatase Calcineurin (Cn). This is a natural coincidence with far-reaching consequences on the immune system: Cn normally dephosphorylates the important immunologic transcription factor NFAT (Nuclear Factor of Activated T-cells), which can then translocate to the nucleus and act as a key regulator of T-cell development and Interleukin-2 production [27•]. CypA/CsA/Cn complexes prevent NFAT dephosphorylation and translocation to the nucleus thus leading to the suppression of the immune system. CsA as a 11mer cyclic peptide displays a 'surface' for binding to the PPIase groove of CypA [28] and one for complexing with Cn (Figure 1). Intensive efforts were made to separate the PPIase blocking from the immunosuppressive functions of CsA. Modifying side chains of the CsA molecule allowed the development of non-immunosuppressive analogs NIM811 [29,30], Alisporivir [ALV, Debio-025] [31], SCY-635 [32], sangliferins [33] and a series of newly synthesized CsA position 1-modified compounds [34–36]. Alisporivir has experienced substantial clinical testing and safety database development with more than 2000 patients treated for up to 48 weeks. NIM811 and SCY-635 have been administered in a very small number (<50 patients) only in short proof-of-concept trials.

Immunophilins and CoV replication

A first hint on the possible involvement of a cyclophilin, namely CypA, in SARS-CoV replication came from an educated guess finding, which demonstrated interaction of the SARS-CoV nucleocapsid (N) protein with CypA by surface plasmon resonance biosensor technology paralleling the binding of HIV-1 gag to CypA [37]. This finding was supported by a proteomics study which identified CypA as one of a number of cellular proteins incorporated into purified SARS-CoV particles by spectrometric profiling [38]. Inhibitory effects of CsA on CoV replication was reported by several laboratories: (1) using unbiased high throughput Y2H protein–protein interaction screening methods we have noticed the binding of several cyclophilins to SARS-CoV nsp1, and CsA as pan-CoV inhibitor including SARS-CoV, HCoV-229E/-NL63, Feline CoV (FCoV) serotypes I and II [strains Black and 791146], Transmissible Gastroenteritis Virus (TGEV PUR46) and Infectious Bronchitis Virus (IBV Beaudette) [16••]. In a follow-up study it could be demonstrated that, at least for replication of HCoV-NL63 CypA, not CypB is the cyclophilin required for virus replication [36]. As also the immunophilins FKBP1A and FKBP1B showed up as nsp1 interaction partners in the Y2H virus–host protein interaction screens mentioned above, SARS-CoV, HCoV-NL63 and HCoV-229E-GFP/-LUC were tested for sensitivity to FK506. The drug could effectively inhibit replication of these viruses, and HCoV-NL63 did not replicate in FKBP1A/B knockdown CaCo2 cell lines [39]. Thus, PPIase activities of CypA and FKBPs seem to be

Figure 1



Crystal structure of human CypA complexed with CsA (1CWA, pdb database, modified) and with coding non-synonymous PPIA gene SNPs. SNPs with accompanying amino acid exchanges introduced in Huh-7.5 PPIA manipulated cell lines [46] are Rs61747111 (D66E), rs17850033 (I89T), rs1059983 (E84D), rs11547706 (G96D), rs17850166 (N106I), rs9769523 (E134K). CypA is shown as a β-sheet structure with the SNP amino acids strongly, or only slightly affecting CoV replication in ball format, colored red and blue, respectively. Active site residues are depicted in orange. CsA binding to the PPIase active pocket of CypA is shown in green. The calcineurin-binding surface of CypA/CsA complex is indicated schematically.