Review

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Cyclophilin inhibition as potential therapy for liver diseases

Nikolai V. Naoumov*

Novartis Pharma AG, Basel, Switzerland

Summary

The cyclophilins are a group of proteins with peptidyl-prolyl isomerase enzymatic activity, localised in different cellular compartments and involved in a variety of functions related to cell metabolism and energy homeostasis, having enhanced expression in inflammation or malignancy. Cyclophilin A (CypA), the most abundantly expressed cyclophilin, is present mainly in the cytoplasm and is a host factor involved in the life cycle of multiple viruses. The extracellular fractions of CypA and CypB are potent pro-inflammatory mediators. CypD, located in mitochondria, is a key regulator of mitochondrial permeability transition pores, and is critical for necrotic cell death. Cyclosporines are the prototype cyclophilin inhibitors. Cyclic peptides, which bind and inhibit cyclophilins without having immunosuppressive properties, have been generated by chemical modifications of cyclosporin A. In addition, cyclophilin inhibitors that are structurally different from cyclosporines have been synthesized. The involvement of cyclophilins in the pathogenesis of different liver diseases has been established using both *in vitro* and *in vivo* investigations, thus indicating that cyclophilin inhibition may be of therapeutic benefit. This review summarises the evidence for potential therapeutic applications of non-immunosuppressive cyclophilin inhibitors, alone or in combination with other agents, in virus-induced liver diseases like hepatitis C, B or Delta, liver inflammation and fibrosis, acetaminophen-induced liver toxicity and hepatocellular carcinoma.

Introduction

Cyclophilins are a group of cellular proteins (collectively known as immunophilins), which display the enzymatic activity of a peptidyl-prolyl isomerase (PPIase) [1,2]. This enzyme catalyses the *cis* to *trans* conversion of proline-containing peptides and facilitates protein folding. Cyclophilins are ubiquitously expressed in all prokaryotic and eukaryotic cells. The first cyclophilin was identified in 1984 as a specific cytosolic protein that binds cyclosporin A (CsA) [3]. However it was not until 5 years later, in 1989, that it was demonstrated that the 18 kDa protein with PPIase activity and cyclophilin (CypA) were in fact the same protein [4,5].

Overall, 17 cyclophilins have been identified in the human genome, however the function of most of these is unknown and only 7 have been characterised for isomerase activity or binding to CsA [1,2]. Cyclophilins share a common domain of approximately 109 amino acids, the cyclophilin-like domain, which is surrounded by domains unique to each member of the family and associated with their subcellular compartmentalization and functional specialization. The subcellular localization of some cyclophilins has been defined, for example – CypA (Cyp18a, where 18 denotes its molecular mass of 18 kDa) and Cyp40 (40 kDa) are present in the cytosol; CypB (22 kDa) and CypC reside in the lumen of the endoplasmic reticulum; CypD – in mitochondria; CypE and CypA are found in the nucleus [1]. CypNK, a 150 kDa molecule, was identified on the surface of human natural killer cells [6].

During the last 20 years considerable knowledge has been accumulated for CypA, CypB, and CypD – concerning their involvement in specific cell functions and disease pathogenesis, while the functional characterization of other members of the cyclophilin family and potential roles in diseases have not been elucidated. CypA is one of the most abundant proteins in the cytoplasm (approximately 0.1% of total cytosolic proteins) and is involved in a range of cellular functions including protein folding, trafficking, immunomodulation and cell signalling [7]. The development of CypA knockout mice and CypA knockdown cell lines has demonstrated that CypA is not essential for cell growth and survival [8,9]. Importantly, CypA is secreted from cells spontaneously and in response to inflammatory stimuli or oxidative stress (reviewed in [10]), and the extracellular fraction of CypA acts as a potent pro-inflammatory mediator, which stimulates inflammatory responses and exerts chemotactic activity for neutrophils and monocytes via the CD147 cell receptor (Table 1). Increased CypA expression has a major role in various pathological conditions such as inflammatory reactions and cartilage destruction in rheumatoid arthritis [18–20]; progression of inflammatory diseases in the lung [24]; exacerbation of oxidative stress and inflammation [21,22]. CypA is also overexpressed in cancer cells and promotes metastasis [27,28].

CypB was the second cyclophilin identified [29]. It differs from CypA mainly by the presence of a cleavable N-terminal sequence

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* Address: Novartis Pharma AG, Novartis Campus, Fabrikstrasse 6, WSJ 157, Basel 4002, Switzerland. Tel.: +41 61 324 9325.
E-mail address: nikolai.naoumov@novartis.com.

Table 1. Key functions of cyclophilin A.

Functions	Target	Biological/pathological effects	[Ref.]
Intracellular			
Protein folding	Collagen	Folding of pro-collagen 1	11
	Transferrin	Folding of transferrin	12
Protein trafficking	Heterogenous nuclear RNP A2	CXCR4-mediated export of hnRNPA2	13
	CD147	Transport to plasma membrane	14
	Asialoglycoprotein receptor (ASGPR)	ASGPR transport between plasma membrane and endosomal pool	15
T-cell activation, cell signaling	Interleukin-2 tyrosine kinase (Itk)	Regulation of T-helper cell profile and cytokines	8,16
	VCAM-1, E-selectin	Proliferation and migration of vascular smooth muscle cells	17
Extracellular			
Stimulates proinflammatory signals	MMP-2, MMP-9 Interleukin-8	Promotes joint inflammation in rheumatoid arthritis, degradation of joint cartilage	18,19,20
Exacerbate oxidative stress	Vascular smooth muscle cells	Oxidative stress	21,22
Chemotaxis of inflammatory cells	CD147/EMMPRIN	Potent leukocyte chemoattractant for human monocytes, neutrophils, eosinophils, and T cells	14,23,24
		Stimulates inflammatory responses when injected *in vivo*	23
		Stimulates expression of adhesion molecules	25,26

hnRNPA2, heterologous nuclear ribonucleoprotein A2; VCAM-1, vascular cell adhesion molecule 1; MMP, metalloproteinase; NF-KB, nuclear factor kappa B; EMMPRIN, extracellular matrix metalloproteinase inducer, or CD147.

that directs the protein to the endoplasmic reticulum. CsA, and other cyclophilin inhibitors, specifically mobilize CypB from the endoplasmic reticulum and promote its secretion from cells, with the extracellular CypB fraction lacking the N-terminal signal sequence [30]. Unlike CypB, CypA is not secreted upon administration of cyclophilin inhibitors. The extracellular fractions of CypA and CypB are involved in cell-cell communications and inflammatory signalling, however on its own, CypB seems unable to induce proinflammatory cytokines [10].

CypD is another key cyclophilin, which is located in the mitochondria and has a central role in regulating the mitochondrial permeability transition pore (MPTP), hence it has been considered as potential therapeutic target for different diseases where mitochondrial dysfunction is central to the disease pathogenesis [31,32]. Mitochondria can be considered as a "firewall" that controls the Ca^{2+} concentration in different cell compartments. Across the outer mitochondrial membrane, the Ca^{2+} transport is mediated mainly by the poorly selective voltage-dependent anion channel (VDAC). Across the inner membrane, the uptake of Ca^{2+} occurs through the mitochondrial Ca^{2+} uniporter and/or the rapid uptake mode, while MPTP has a major role for Ca^{2+} efflux from mitochondria to the cytosol [33]. When opened in a flickering mode, the MPTP acts as a fast Ca^{2+} release channel, generating waves of cytosolic Ca^{2+} that propagate signals to other cell regions or are taken up by surrounding mitochondria. CypD is localized in the matrix of mitochondria and regulates MPTP opening and consequently the Ca^{2+} exchange between the mitochondria and the cytosol (Fig. 1). Several lines of evidence from experiments using hepatocytes, neurons or cardiomyocytes have established that CypD, is the key regulator of MPTP opening [32,33]. A persistent MPTP opening induces necrotic cell death, which is different from necroptosis – a regulated cell necrosis

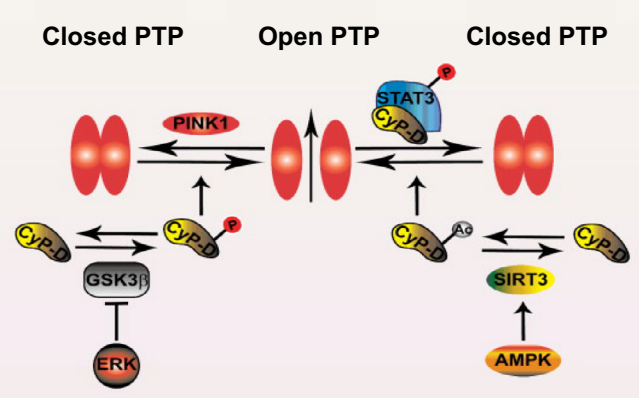

Fig. 1. Cyclophilin D (CyP-D) regulates opening of the mitochondrial permeability transition pore (PTP). (i) CyP-D phosphorylation by GSK3 facilitates PTP opening; (ii) phosphorylated STAT3 binds to CyP-D and inhibits PTP opening; (iii) CyP-D acetylation sensitizes the PTP to opening and is prevented by AMPK activation of the SIRT3 de-acetylase. Reprinted from [33] © 2014, with permission from Elsevier.

that is dependent on the receptor-interacting protein kinase 3, RIPK3 [34]. Recently, it has been shown that cell necrosis, as a result of mitochondrial permeability transition or necroptosis, occurs via two co-existing but separate pathways, and the combined blockade of both pathways, – such as CypD inhibition with cyclosporine or a non-immunosuppressive Cyp inhibitor (sanglifehrin A) together with necroptosis inhibition with the RIPK1 inhibitor necrostatin-1, has resulted in strong and additive protection from ischemia-reperfusion injury [34,35]. CypD plays a