Biochimica et Biophysica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

Extracellular cyclophilins in health and disease ☆

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ARTICLE INFO

Article history:
Received 29 September 2014
Received in revised form 11 November 2014
Accepted 14 November 2014
Available online xxxx

Keywords:
CD147
Extracellular cyclophilin
Cyclosporine A
Disease model
Chemotaxis
Inflammation

ABSTRACT

Background: Extracellular cyclophilins (eCyPs) are pro-inflammatory factors implicated in pathogenesis of a number of inflammatory diseases. Most pathogenic activities of eCyPs are related to their chemotactic action towards leukocytes, which is mediated by eCyP receptor on target cells, CD147, and involves peptidyl–prolyl *cis–trans* isomerase activity of cyclophilins. This activity is inhibited by cyclosporine A (CsA) and non-immunosuppressive derivatives of this drug. Accumulating evidence for the role of eCyPs in disease pathogenesis stimulated research on the mechanisms of eCyP-initiated events, resulting in identification of multiple signaling pathways, characterization of a variety of effector molecules released from eCyP-treated cells, and synthesis of CsA derivatives specifically blocking eCyPs. However, a number of important questions related to the mode of action of eCyPs remain unanswered.

Scope of review: In this article, we integrate available information on release and function of extracellular cyclophilins into a unified model, focusing on outstanding issues that need to be clarified.

Major conclusions: Extracellular cyclophilins are critical players in pathogenesis of a number of inflammatory diseases. Their mechanism of action involves interaction with the receptor, CD147, and initiation of a poorly characterized signal transduction process culminating in chemotaxis and production of pro-inflammatory factors.

General significance: Extracellular cyclophilins present an attractive target for therapeutic interventions that can be used to alleviate symptoms and consequences of acute and chronic inflammation. This article is part of a Special Issue entitled Proline-directed Foldases: Cell Signaling Catalysts and Drug Targets.

1. Introduction

Cyclophilins are ubiquitously expressed intracellular proteins first recognized as the host cell receptors for the potent immunosuppressive drug, cyclosporine A [1]. Later, it was established that cyclophilins and previously identified peptidyl–prolyl isomerases, which are characterized by their ability to catalyze the interconversion of *cis* and *trans* isomers of proline, are the same proteins [2,3]. The family of human cyclophilins includes seventeen identified members [4]. Cyclophilins are highly abundant proteins (for example, CyPA is present in cells in micromolar concentrations) that are localized to a variety of cellular organelles and subcellular compartments, suggesting that they may perform important cellular functions. It was therefore surprising that knockout of CyPA had no impact on cell viability or the life span of knock out animals [5]. Intracellular functions, in most cases related to the isomerase activity, have been characterized for only a small number of cyclophilins (see a recent review by Hoffmann and Schiene-Fischer [6]). This deficiency is due to our very limited knowledge of proteins that interact with cyclophilins, mostly because of the transitory nature of such interactions characteristic to enzymatic reactions. Interestingly, several members of the human cyclophilin family (RanBP2, SDCCAG-10, and cyclophilin-like proteins PPIL2 and PPIL6) were found to lack isomerase activity and did not bind CsA [4], probably reflecting redundancy within this family of molecules and their long evolutionary history.

The first cyclophilin to be found in extracellular fluids was cyclophilin B (CyPB), an abundant protein localized to ER due to the N-terminal signal sequence, which was identified in human milk and was initially described as a secreted protein [7]. A later study from the same group suggested that secreted CyPB is a C-terminal truncation of the protein that lacks a portion of the unconventional C-terminal ER retention signal [8]; however, the protease responsible for this cleavage or the mechanisms regulating this process under physiological or pathological conditions have not been reported. Moreover, a recent report characterized the ER retention signal in CyPB as overlapping with the CsA-binding site, which is located away from the C-terminus, and demonstrated that CsA treatment or mutation of the ^{128}Tryptophan in the CsA-binding site released CyPB into the extracellular milieu [9]. Given that CyPB has been found as a component of an extensive network of ER chaperones and folding catalysts that include BiP, Grp94, Grp170, co-chaperone ERdj3, and members of the protein disulfide isomerase (PDI) complex ERp72, P5, PDI, calnexin and calreticulin [10,11],

☆ This article is part of a Special Issue entitled Proline-directed Foldases: Cell Signaling Catalysts and Drug Targets.

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http://dx.doi.org/10.1016/j.bbagen.2014.11.013
0304-4165/

interaction with these ER resident proteins via the CsA-binding site may determine the ER localization of CyPB and explain the secretion of this protein after CsA treatment. It remains unclear how secretion of CyPB is regulated when no CsA is present.

In 1992 a surprising and initially underappreciated finding was reported that cyclophilin A, an abundant cytosolic protein, is secreted by macrophages in response to LPS stimulation [12]. The mechanisms of CyPA secretion have remained mysterious for a long time, as this protein lacks any known export signal. Later studies demonstrated that CyPA secretion is regulated by a vesicular mechanism dependent on Rho activation [13], consistent with previous reports of CyPA release from cells subjected to different types of stress [12,14,15]. Recent proteomics analysis of CyPA released from irradiated breast cancer cells suggested post-translational modifications of the secreted cyclophilin [16], a finding confirmed in studies performed on smooth muscle cells treated with oxidation stress-inducing agent angiotensin II [17]. Here, specific acetylation on lysine residues 82 and 125 was found necessary for secretion and further activity of CyPA on endothelial cells. Consistent with previous studies showing Rho-dependent secretion of CyPA, acetylation of CyPA was also dependent on Rho, supporting the essential role of acetylation in the process of CyPA secretion, at least in the case of smooth muscle cells subjected to oxidative stress. It remains to be determined how widespread this pattern of secretion is and whether it involves other secreted cyclophilins (CyPB and CyPC). Given that constitutive secretion of CyPA and CyPB has been described for certain cell types (CyPA secretion from fibroblast-like synoviocytes [18], head and neck/oral squamous carcinoma cells [19], and adipocytes during differentiation [20], and CyPB secretion from chondrocytes [21] and pancreatic cancer cells [22]) it will be also important to determine the ratio of acetylated and non-acetylated cyclophilins, in particular CyPA, in various cell types and under various activation stimuli. Indeed, if acetylation marks the CyPA molecules destined for secretion, knowledge of the proportion of these molecules in the whole pool of intracellular CyPA in a particular cell type under specific treatment conditions (activation, infection, etc.) would provide an estimate of potential contribution of such cells to the inflammatory response and may identify them as a target for therapeutic interventions.

The third member of the cyclophilin family that can be secreted from cells is CyPC. Secretion of this protein was detected by proteomics approaches in pre-adipocytes undergoing differentiation to adipocytes [20] and unstimulated rat leptomeningeal cells [23]. Recent study demonstrated that, similar to CyPB, CyPC localizes to the ER and is released to the extracellular space by CsA treatment [24]. It remains to be determined whether extracellular CyPC plays any specific physiological or pathological role.

While initial studies have focused on the intracellular activities of cyclophilins, accumulating evidence suggests a role for these proteins as mediators of intercellular communications [25]. Two excellent recent reviews provided a comprehensive description of extracellular cyclophilins, their functions and activities [6,26]. In this article, we attempted to integrate available information on these medically important molecules into a unified model while focusing on the unresolved issues and controversies remaining in the field (Table 1).

2. Functions of secreted cyclophilins

Secretion of cyclophilins has been identified after stimulation with pro-inflammatory or oxidative agents, or by proteomics approaches that did not include functional analysis. Therefore, very little experimental information is available regarding the physiological role of extracellular cyclophilins, and this issue remains open to speculations. Some conjectures regarding potential functions of extracellular cyclophilins can be made from studies performed with knockout mice, although this model does not allow definitive discrimination between the effects of intracellular and extracellular cyclophilins. Initial analysis of CyPA knockout mice did not reveal any major defects, and the life span of the animals was the same as wild-type littermates, leading to a conclusion that CyPA is not essential in mammals [5]. This result is quite surprising given the high level expression of CyPA in all tissues [27], and suggests that the role of CyPA, including the extracellular

Table 1
Main unresolved issues related to extracellular cyclophilins.

Issue	What is known?	What is unknown?	References
Receptor for eCyPs	CD147 is essential for most known effects of eCyPs; CyPA catalyzes *cis–trans* isomerization of the Trp^{210}–Pro^{211} bond of CD147; CD147 interacts with signal transducing receptors, such as CD98 and integrin β1.	Is there any alternative receptor to eCyPs? Is there any difference between eCyPA and eCyPB in their interaction with CD147? Is isomerization of CD147 responsible for signal transduction and how are signals generated? What CD147-interacting molecules are involved in signal transduction initiated by eCyPs?	[47,6,54,55,28]
Signaling induced by eCyPs	eCyPs initiate CD147-dependent Ca^{2+} flux, ERK1/2 and JNK activation.	What are signal transduction pathways activated by eCyPs? Are there differences in signaling initiated by eCyPA, eCyPB and eCyPC?	[6,65–70]
Post-translational modifications of eCyPs	Acetylation of CyPA promotes its secretion and functional activity on endothelial cells.	Are acetylation or other post-translational modifications necessary for secretion of all eCyPs from all cell types? What is the ratio of modified and unmodified cyclophilins in various cell types? Do post-translational modifications alter interaction with CD147 and functional activity of eCyPs?	[16,17]
Mechanism of eCyP secretion	CyPA is secreted via a vesicular mechanism dependent on Rho activation.	How is secretion of eCyPs regulated? What is the mechanism of secretion of eCyPB and eCyPC?	[13,16]
Functional activities of eCyPs	eCyPA is a chemotactic agent for leukocytes; eCyPB stimulates adhesion of memory T cells to extracellular matrix; eCyPA stimulates production of ROS by VSMC and MMP and pro-inflammatory cytokines by myeloid cells	What are the physiological functions of eCyPs? What is the role of eCyPs in pathology of inflammatory and other diseases?	[37,49,40,41,75,25]
eCyPs as a therapeutic target	Blockade of eCyPs or CD147 ameliorates inflammation and disease in models of rheumatoid arthritis, asthma, biliary atresia; Atherosclerosis is reduced in $CyPA^{-/-}$ mice.	What other diseases can be treated by targeting eCyPs? How does treatment targeting eCyPs or CD147 compare to currently used treatments of inflammatory diseases (e.g., anti-TNFα)? Can non-permeable CsA derivative with extended half-life time be created?	[28,77,64,78,79]

Please cite this article as: M. Bukrinsky, Extracellular cyclophilins in health and disease, Biochim. Biophys. Acta (2014), http://dx.doi.org/10.1016/j.bbagen.2014.11.013