Research Article

Procollagen C-proteinase enhancer-1 (PCPE-1), a potential biomarker and therapeutic target for fibrosis

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Abstract

The correct balance between collagen synthesis and degradation is essential for almost every aspect of life, from development to healthy aging, reproduction and wound healing. When this balance is compromised by external or internal stress signals, it very often leads to disease as is the case in fibrotic conditions. Fibrosis occurs in the context of defective tissue repair and is characterized by the excessive, aberrant and debilitating deposition of fibril-forming collagens. Therefore, the numerous proteins involved in the biosynthesis of fibrillar collagens represent a potential and still underexploited source of therapeutic targets to prevent fibrosis. One such target is procollagen C-proteinase enhancer-1 (PCPE-1) which has the unique ability to accelerate procollagen maturation by BMP-1/tolloid-like proteinases (BTPs) and contributes to trigger collagen fibrillogenesis, without interfering with other BTP functions or the activities of other extracellular metalloproteinases. This role is achieved through a fine-tuned mechanism of action that is close to being elucidated and offers promising perspectives for drug design. Finally, the *in vivo* data accumulated in recent years also confirm that PCPE-1 overexpression is a general feature and early marker of fibrosis. In this review, we describe the results which presently support the driving role of PCPE-1 in fibrosis and discuss the questions that remain to be solved to validate its use as a biomarker or therapeutic target.

Introduction

Fibrillar collagens are the most abundant proteins in the human body and their biosynthesis is a critical pathway controlling development, growth, homeostasis and aging. Fibrillar collagens and the numerous regulators of their synthesis and degradation also represent potential targets in several diseases, especially those derived from altered collagen turnover. The latter include but are not limited to defective tissue repair [1,2], cancer [3] and osteoarthritis [4]. Here, we focus on fibrosis, a general term referring to the common pathogenic outcome of a great diversity of uncontrolled tissue repair responses leading to the aberrant accumulation of extracellular matrix (ECM) [5]. Collagens are the most abundant components of fibrotic lesions and they are known to be responsible for tissue stiffening and impaired function, potentially leading to death when a vital organ is affected. Despite the intense efforts of the scientific community to identify relevant and druggable protein targets to alleviate the deleterious effects of fibrosis, very few drugs have demonstrated an efficient and direct anti-fibrotic activity [5]. As a key regulator of collagen fibrillogenesis, the protein named procollagen C-proteinase enhancer-1 (PCPE-1) attracts more and more attention. PCPE-1 is a

potent and specific enhancer of fibrillar collagen proteolytic maturation, the rate-limiting step in collagen fibrillogenesis. In this review, we show that in addition to the prominent role of PCPE-1 in collagen fibrillogenesis, its specific mechanism of action, its expression pattern, which closely matches that of collagen I, and the fact that it is relatively more dispensable to tissue homeostasis than most other players in collagen synthesis, also make it a very promising target for the treatment or prevention of fibrotic disorders.

PCPE-1 in collagen fibrillogenesis

Fibrillar collagens are usually classified into "major" (collagens I, II and III) and "minor" (collagens V and XI) collagen types based on their relative abundance. The common view is that minor fibrillar collagens co-assemble with major fibrillar collagens to regulate fibril growth [6,7]. This classification does not take into account the more recently discovered members of the fibril-forming collagens (collagens XXIV and XXVII), which exhibit a more restricted expression pattern and produce fibrils with distinct characteristics [8,9]. Collagens I, V and XI form heterotrimers composed of two to three different polypeptide (α) chains while collagens II and III form homotrimers composed of three identical chains [10,11]. With the notable exception of normal cartilage ECM which mainly consists of fibrils made of collagens II and XI, the ECM of connective tissues is usually composed of heterotypic fibrils where collagens I, III and V are present in various proportions [10]. Collagen I is the major fibrillar component while collagen III is most abundant in blood vessels and collagen V in the cornea [7,12]. All three collagen types are interdependent and the lack of one collagen has broad consequences on fibrillogenesis [13,14].

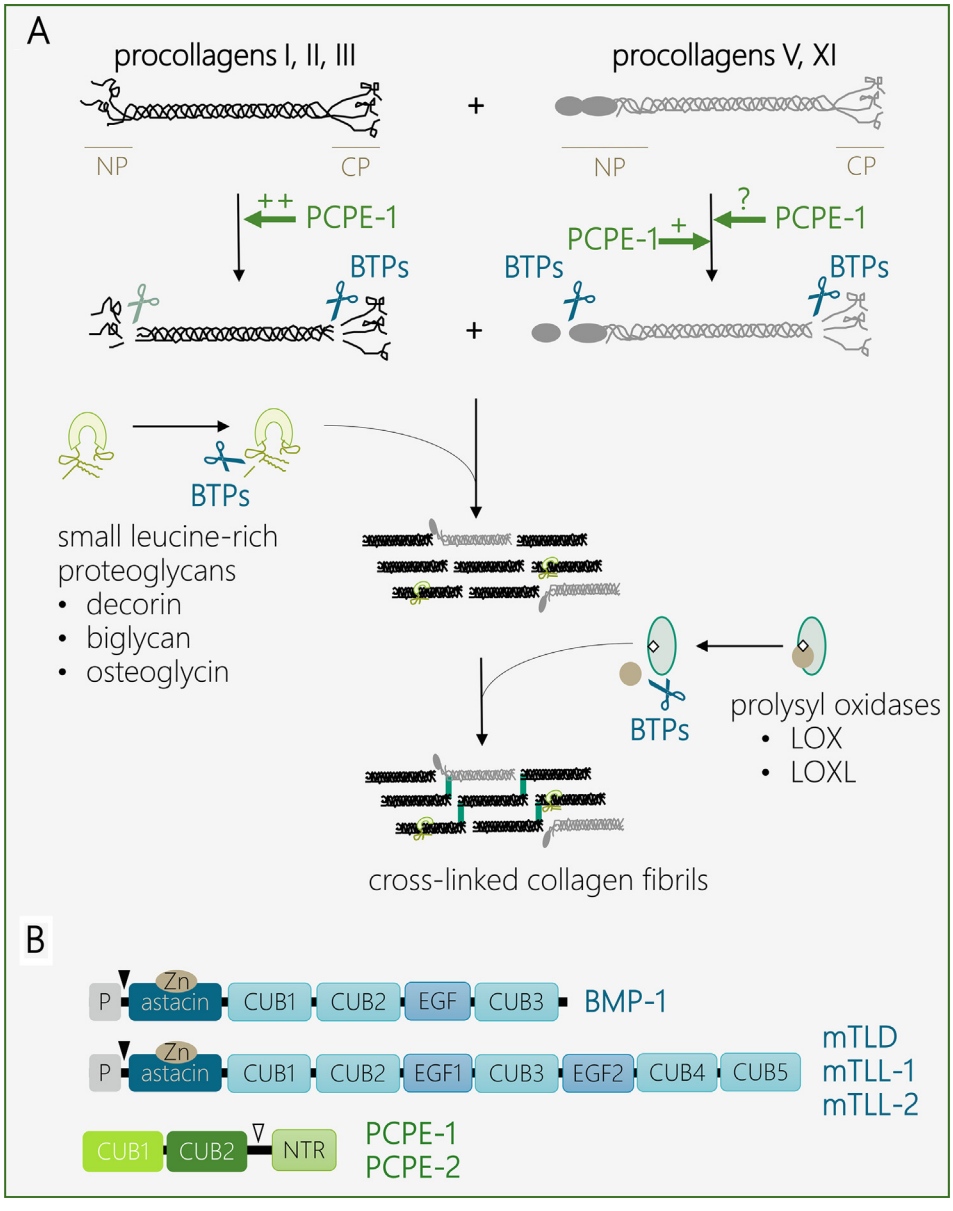
The various steps leading from individual collagen chains to fibrils are well described for major and minor collagens [15]. This pathway starts with the synthesis of collagen precursor molecules, also known as procollagens, which then undergo several post-translational modifications (e.g. proline and lysine hydroxylations) and assemble into trimers. At this stage, trimeric procollagen molecules are kept in soluble form through their charged N- and C-terminal domains called propeptides. During or after secretion into the extracellular environment, the propeptides are cleaved by specific proteolytic enzymes and fibrillogenesis can occur (Fig. 1A). Fibril formation is supposed to be a spontaneous process, as observed *in vitro* [16], but several additional protein partners are also involved *in vivo* to obtain the specific fibril characteristics that best fit to collagen tissue function. Among these partners, FACITs (fibril-associated collagens with interrupted triple helices) and SLRPs (small leucine-rich proteoglycans) play major roles by regulating fibril diameter, spacing and interactions [7,17]. The final step of fibrillogenesis is the stabilization of the collagen network by the formation of intermolecular cross-links, a process initiated by lysyl oxidases LOX and LOXL [18].

The proteolytic enzymes that cleave procollagen propeptides are known as procollagen N- and C-proteinases (PNPs and PCPs). Ten different proteinases, belonging to the astacin, ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) and SPC (subtilisin-like proprotein convertase) subgroups, can cleave at least one propeptide in at least one fibrillar collagen [19–22]. Of specific interest here are the bone morphogenetic protein-1 (BMP-1)/tolloid-like proteinases or BTPs [22]. They form a group a four astacin-like proteinases comprising BMP-1 and mTLD (mammalian tolloid), two splicing variants of the same *BMP1* gene, and mTLL-1 and -2 (mammalian tolloid-like 1 and 2) which are encoded by two additional genes (*TLL1* and *TLL2*). These are the main enzymes in charge of the C-terminal maturation of fibrillar collagens [23], the most important proteolytic event to trigger fibrillogenesis [16] (Fig. 1A). They can also cleave the N-termini of minor fibrillar procollagens V and XI, releasing the first thrombospondin-like N-terminal (TSPN) domain of their α1 chain but leaving most of their N-propeptide attached to the main triple helix [21,24–26]. In addition, they process the SLRPs decorin, biglycan and mimecan/osteoglycin by removing a short sequence at their N-terminus [27–29]. Both partial collagen V and XI N-propeptide retention and SLRP binding to fibrils are recognized as key mechanisms to control fibril lateral growth [7]. Finally, BTPs process the lysyl oxidase members LOX and LOXL [30–32], thereby promoting their activation and the formation of covalent cross-links between collagen monomers (Fig. 1A).

PCPE-1 was first described by Adar and colleagues in 1986 as a contaminant in PCP preparations [33] where they observed that it was capable of significantly increasing procollagen I C-terminal processing. For this reason, the name procollagen C-proteinase enhancer (PCPE) is most often used while the other commonly found abbreviation PCOLCE is kept for the gene name. *PCOLCE* is localized to the same region of chromosome 7 that contains the *COL1A2* gene [34] and leads to a 50 kDa glycoprotein composed of two N-terminal CUB (complement, Uegf, BMP-1) domains and one netrin-like (NTR) domain, separated by a long linker (Fig. 1B). Homologues of PCPE-1 are rather ubiquitous in vertebrates and their amino acid sequences are well conserved with the exception of the linker sequence [34–36]. In human and mouse proteins, this linker is prone to proteolysis and can be cleaved *in vitro* by a wide range of proteases including trypsin [37], plasmin [38], MMP2 [39] and meprins [40]. Thus, proteolytic fragments corresponding to both CUB1CUB2 and NTR domains

have been detected *in vivo* and in cell culture [33,38,41,42]. In addition, proteolysis of human PCPE-1 within CUB1 was reported under the action of soluble ADAM17 [43] but this cleavage seems less predominant.

Interestingly, PCPE-1 is very specific to procollagen maturation and was found to stimulate the C-terminal processing of procollagens I, II and III by BTPs [33,44,45]. There are some hints that it could also promote the C-terminal maturation of the α2 chain of procollagen V in the supernatant of mouse embryonic fibroblasts [46] but this has never been confirmed *in vitro* and is theoretically not possible if the α2 chain is included in a heterotrimer, as will be discussed below. In contrast, the N-terminal removal of the TSPN domain of the procollagen V α1 chain by BTPs was shown to be enhanced by PCPE-1 [47], although some less conclusive data had first been obtained, probably because of the presence of contaminating PCPE-1 in the procollagen preparation [44]. The extent of stimulation seems somewhat lower than for C-terminal processing events, although this has not been precisely quantified. Also, the mechanism of N-terminal enhancement is not well understood for the moment and it will not be discussed further here. Specificity to procollagen maturation is however a very striking feature of PCPE-1 and, despite the ever-growing list of reported BTP substrates [22,48–50], no other cleavage event seems to be affected by PCPE-1. The lack of effect of PCPE-1 was thus demonstrated for other BTP substrates involved in collagen fibrillogenesis such as osteoglycin and LOX but also for other ECM components processed by BTPs such as procollagen VII, laminin 332, dentin matrix protein-1 and dentin



sialophosphoprotein or for growth factor antagonists which are inactivated by BTPs such as chordin [44,51,52].

PCPE-1 expression is high in tissues rich in collagen I such as bones, tendons, skin or cornea and its abundance is lower when the collagen content is less dominant [34,37,53,54]. In early studies, this protein was not detected in brain and liver and it was barely detected in heart and kidneys [37] but this was tempered in later studies which showed that *PCOLCE* RNA and its derived protein were relatively high in the heart [45,55]. Interestingly and in contrast to what is described for *Bmp1*- and *Tll1*-null mice which die around birth or even before [25,56,57], *Pcolce*-null mice are viable, fertile and do not show any obvious defects [46]. However, a closer inspection of bones and tendons revealed that their collagen fibrils were abnormal with irregular and fluted shapes [46]. In the left femora that were analysed by transmission electron microscopy, some broadening and branching of fibrils was also observed. This results in macroscopic alterations of cortical and trabecular bone geometries which are explained as a compensatory mechanism for inferior material properties [46]. The importance of the *Pcolce* gene for efficient tissue repair was also demonstrated in several models of corneal injury, all of which were characterized by a more or less pronounced delay in wound closure in KO mice [54]. These results will be discussed in more detail below but, overall, they seem to relate to a relatively mild phenotype compared to the effect of mutations in genes encoding collagen chains, BMP-1 or other enzymes involved in collagen synthesis [58]. Notably, no human genetic disease seems to be associated with mutations in *PCOLCE*.

Another human PCPE protein has been described in 2000 which shares 43% identity with PCPE-1 and has the same domain composition (Fig. 1B). It was called PCPE-2 and the corresponding gene *PCOLCE2* [45,59]. From the few studies that have analysed PCPE-2 activity, it seems to be endowed with the ability to increase the C-terminal processing of procollagen II by BMP-1 [45] but less clearly of procollagen I [60]. PCPE-2 has also been linked to high density lipoprotein (HDL)-cholesteryl ester uptake and atherosclerosis [61,62]. Finally, it was found that *Pcolce2*-null mice are protected against cardiac fibrosis in a model of transverse aortic constriction [63]. However, since the data about its PCP or PNP enhancing activity and its possible involvement in fibrosis are scarce, we have decided to focus here on PCPE-1.

Structure and mechanism of action of PCPE-1

PCPE-1 is a secreted protein containing multiple post-translational modifications [34,64]. It is decorated with sialylated O- and N-glycosylations that may differ in healthy and diseased states, resulting in a complex migration pattern in isoelectric focusing (IEF) experiments [45,65,66]. Two N-glycosylation sites are predicted but only the most N-terminal one seems functional, its absence inducing lower protein stability [67] but having no impact on PCPE-1 activity [68]. The mature protein also contains 14 cysteines and correct disulfide bonding is necessary for activity [69]. Other modifications such as phosphorylations or myristoylations have been detected or predicted [59,70] and may contribute to the complex pattern observed in IEF.

PCPE-1 is a monomer in solution and the first low-resolution structural data showed an elongated molecule of about 150 Å in length [71]. This finding was somewhat unexpected since CUB and NTR domains have very different isoelec-

Fig. 1. Main extracellular steps of collagen fibril formation and domain structure of BTPs and PCPEs. (A) After secretion, procollagens remain soluble until they are processed by N- and C-proteinases which remove their N- and C-propeptides (NPs, CPs). The BTPs play a major role in these maturation steps as they are in charge of cleaving the C-propeptides of collagens I, II and III. They can also process the C-terminus of the α1 and α2 chains of procollagens V and XI, although SPCs might act before on the α1 chains. Finally, BTPs mature the N-terminus of the α1 chain of procollagens V and XI but this proteolytic event preserves a large part of their N-propeptide which, after incorporation into fibrils, protrudes from the fibril surface and inhibits further fibril lateral growth. PCPE-1 enhances the C-terminal maturation of collagens I, II and III and, to a lesser extent, the N-terminal maturation of the α1 chain of collagen V. BTPs control other aspects of collagen fibrillogenesis through the proteolytic maturation of decorin, biglycan and osteoglycin (SLRPs) which interact with collagen fibrils and regulate their diameter and spacing. They also activate the lysyl oxidases LOX and LOXL, thereby triggering the formation of cross-links between collagen monomers to increase fibril stability. (B) Domain composition of the BTPs and PCPEs. BMP-1 and mTLD are splice variants differing by the presence of a short C-terminal sequence in BMP-1 and of one additional EGF and two additional CUB domains in mTLD. mTLL-1 and mTLL-2 domain structures are similar to that of mTLD. All BTPs are synthesized as proenzymes and are activated by SPCs (site indicated by arrowhead) in the secretory pathway. The catalytic activity is conferred by the astacin-like domain and other domains contribute to regulate substrate specificity. PCPE-1 and PCPE-2 have the same domain composition with two N-terminal CUB domains and a C-terminal NTR domain connected by a long linker. The CUB domains of PCPE-1 can be proteolytically released (site indicated by arrowhead) and retain full enhancing activity *in vitro*. P = propeptide.

tric points (around 5 for individual CUB domains and 9.5 for NTR) whereas the full-length protein is rather neutral [65,69], suggesting possible interactions between the two ends of the molecule.

The original purification protocol of PCPE-1 from mouse fibroblast supernatants [33,64] involved the use of a Sepharose column coupled to the C-propeptide of procollagen I (CPI). PCPE-1 was co-eluted from this column with the so-called "PCP", already suggesting a strong affinity for CPI and/or the protease. Interestingly, although this procedure also enabled the co-purification of a shorter 36 kDa fragment (corresponding to the CUB domains, Fig. 1B), only the latter and not the full-length PCPE-1 was separated from the protease by size exclusion chromatography [33,64,72]. This was the first indication that the NTR domain of PCPE-1 was probably necessary for the interaction of the enhancer with the protease, a finding which was later confirmed by surface plasmon resonance experiments [73] even though the interaction remains weak (K_D > 100 nM).

PCPE-1 is devoid of catalytic activity on its own but, as already mentioned above, is able to specifically stimulate CP removal from fibrillar procollagens I, II or III [44,45,64] by all four BTPs [25,45,74,75]. PCPE-1 has nanomolar affinity for procollagen molecules [76–78] and kinetic studies have shown that it can increase the catalytic efficiency of BMP-1 by a factor of 15 on procollagen I and by a factor of 12 on a mini-procollagen III [68,79]. Of note, this increase in catalytic efficiency derives from both the increased affinity of the enzyme for the procollagen substrate and the increased turnover number of the hydrolysis reaction.

Several studies demonstrated that the enhancing activity of PCPE-1 is provided by its CUB domains [33,64,75,76] and is due to a direct and strong calcium-dependent interaction with the procollagen CPs (K_D < 30 nM) [75–78]. Through mutagenesis studies with deletion and domain-swapping mutants, it was then observed that contiguous CUB1 and CUB2 domains are both necessary and sufficient for PCPE-1 activity [76]. This led to the isolation of the complex formed by CUB1CUB2 and CPIII which was first analysed by small angle X-ray scattering (SAXS) [68]. The derived low-resolution structure of the complex revealed that CUB1CUB2 binds to the N-terminal/"stalk" region of CPIII [80], close to the BTP cleavage site, and confirmed the previously evidenced and intriguing 1:1 (PCPE-1 monomer:procollagen trimer) stoichiometry [35,69,77]. The recent crystal structure of the same complex [81] gave additional information and unveiled the key determinants of the mechanism of action of PCPE-1 (Fig. 2A). It shows that a single PCPE-1 molecule winds around the CPIII trimer with each CUB domain interacting mainly with a specific procollagen chain (e.g. in Fig. 2, CUB1 with chain A and CUB2 with chain B). The complex is stabilized around the two calcium binding sites of CUB1 and CUB2 through interactions with two conserved and distinct CP lysines (one in each procollagen chain; upper left inset in Fig. 2A) that are essential for PCPE-1 binding, and therefore, activity [35,68,81]. Importantly, this mode of action mobilizes two procollagen chains simultaneously, precluding the binding of a second PCPE-1 molecule. Also, it is interesting to note that the conserved lysines are found in all chains of major fibrillar procollagens and in the α2 chain of procollagen V but not in other chains of minor fibrillar procollagens [80], making it unlikely that PCPE-1 modulates their C-terminal maturation by BTPs. However, it cannot be excluded that an alternate mechanism is at play for these collagens.

The structure also shows that upon CUB1CUB2 binding, the stalk region of the CPIII trimer is distorted, with the end of chain A being "pulled" into the groove between the CUB domains. The BMP-1 active site being too small to accommodate more than one procollagen chain [82], this loss of symmetry offers two possibilities for the protease to bind to the procollagen/PCPE-1 complex. Separated from chain A, chain B or C could become more accessible to enter the enzyme active site (Fig. 2B and C) or the BMP-1 catalytic domain could bind directly to the locally unraveled chain A (Fig. 2A). Molecular modelling favors this last mechanism since it would create a new interaction surface between PCPE-1 and the protease [81] and contribute to the previously observed decrease in K_M [68,79]. Once the first chain is cleaved, the sequential cleavage of the remaining chains is probably facilitated, independently of the presence of PCPE-1.

It should be noted that the current model does not include BMP-1 or tolloid non-catalytic domains. Their exact role remains to be determined but BMP-1 CUB and EGF domains have been shown to be necessary for full enhancement by PCPE-1 ([51,52,74] and our unpublished results). This implies that a direct interaction between PCPE-1 and the protease is involved at one stage of the catalytic process. If the interaction between BMP-1 and PCPE-1 is actually weak for the full-length enhancer and null for CUB1CUB2 [68,73,74], it is probably strengthened in the presence of the procollagen molecule even if the exact details of this three-partner interaction are presently unknown. The involvement of the protease auxiliary domains is also supported by the fact that PCPE-1 does not enhance the cleavage of procollagen III by another family of PCPs, the meprins [20,40], that have a similar astacin-like catalytic domain but no CUB or EGF domains [40]. Instead, PCPE-1 inhibits meprin-mediated release of CPIII, most probably because its binding to procollagen III hinders protease access to the cleavage site.

The role of the NTR domain of PCPE-1 is less directly connected to the enhancing mechanism

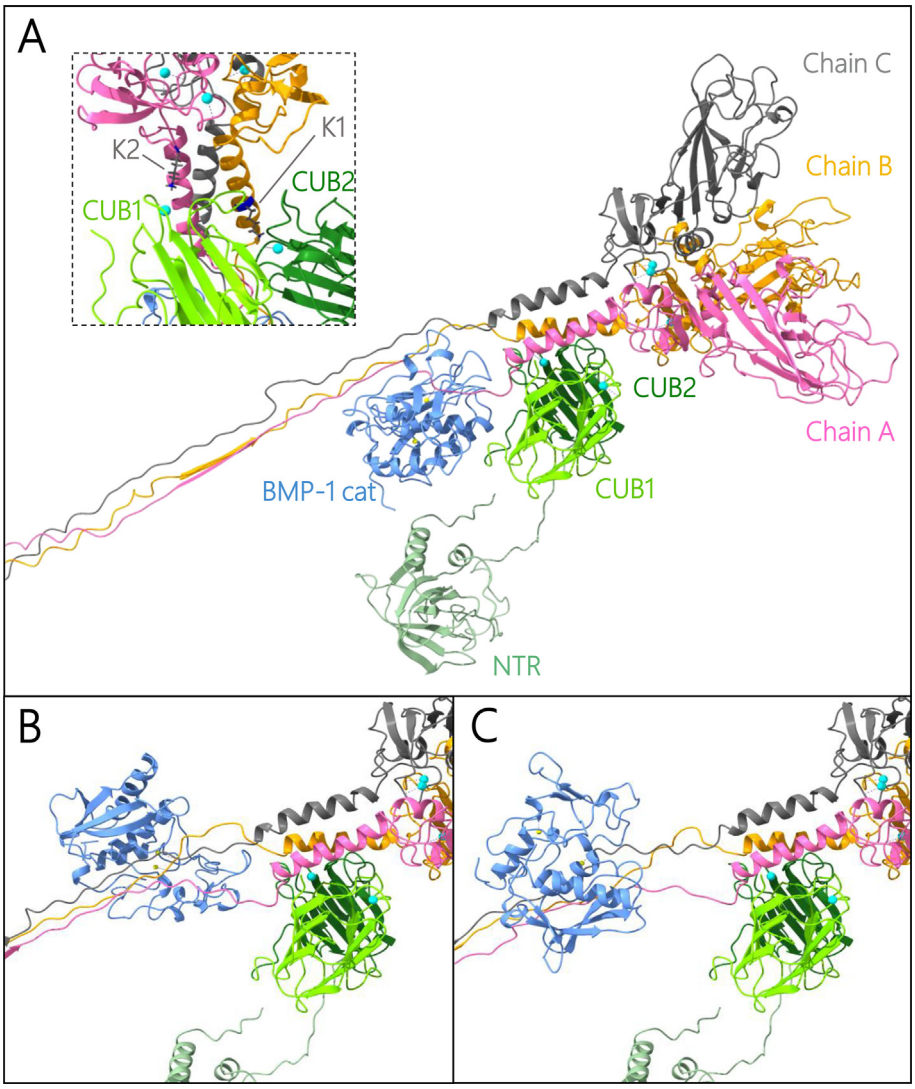


Fig. 2. Current models of the procollagen C-terminal maturation complex. Starting from the crystal structure of CPIII in complex with the CUB1CUB2 region of PCPE-1 (PDB code: 6FZW; [81]), modelling was used to extend chains A (in pink), B (in yellow) and C (in grey) of procollagen III towards the C-telopeptide region and the collagen triple helix (see [81] for details). Full-length PCPE-1 (in green) was rebuilt using YASARA (http://www.yasara.org) for homology modeling, based on Uniprot/Swiss-Prot sequence Q15113, PDB code 6FZW for CUB1CUB2 and PDB code 1UAP for the NTR domain. YASARA was also used to successively model the BMP-1 catalytic domain (BMP-1 cat; PDB code: 6BSL, in blue) in interaction with chain A (A), chain B (B) or chain C (C) of procollagen III. Cyan spheres represent calcium ions and yellow spheres are zinc ions. Rendering was obtained using UCSF ChimeraX [178]. In (A), the inset shows a close-up view of the interface between PCPE-1 and procollagen III. The two procollagen lysines (K1 and K2) involved in the interaction are highlighted in grey. As discussed in the text, the model shown in (A) is the most likely. Note that the NTR domain of PCPE-1 is shown to give a full view of the protein and an idea of the volume occupied by this domain but it is not known how it could interact with other partners in the complex. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

but is still very important. This domain is homologous to tissue inhibitors of metalloproteinases (TIMPs) [83] and it can be released proteolytically. However, an early report suggesting that it was a weak inhibitor of matrix metalloproteinases [38] was not confirmed by later studies [36,73]. In contrast, the NTR domain can strongly interact with heparin and heparan sulfate proteoglycans (HSPGs), i.e. syndecans-1, -2 and -4, and mediates PCPE-1 attachment to the cell surface [69,73,84,85]. *In vitro*, the presence of heparin induces a “super-stimulation” of procollagen processing, through the increase of PCPE-1 enhancing activity (around two-fold), but only when the NTR domain is attached to the CUB domains [73]. Since procollagens and BTPs are also able to interact with HSPGs, PCPE-1 may contribute to forming large protein complexes at the cell surface or in the

matrix, where anchoring through its NTR domain would allow optimal orientation of its CUB domains and maximal activity. In line with this, PCPE-1 was also found to interact with fibronectin [84], fibulin-4 [86] and thrombospondin-1 [87], all of which are involved in collagen supramolecular maturation.

Enhancement of PCP activity could also be amplified in the presence of secreted frizzled related protein 2 (sFRP2). sFRP2 has been described as another enhancer of procollagen I cleavage by BTPs [88] and, even if the activity of this protein alone remains highly controversial [52,89,90], it was recently reported that sFRP2 is able to interact with mouse PCPE-1 and that both proteins could act in synergy to increase procollagen processing *in vitro* [91]. Indeed, while *Sfrp2* knock-out mice are viable and do not show any major defects [92], the simultaneous knockdown of *Sfrp2* and *Pcolce* is embryonic lethal and cultures of mouse embryonic fibroblasts derived from these mice show a significant decrease in collagen maturation. Similarly, double knockdown of *Sfrp2* and *Pcolce* in zebrafish led to a strongly dorsalized phenotype with decreased procollagen processing and survival limited to 72 h. The mechanism is unclear but seems to involve the CUB domains of PCPE-1 and the frizzled domain of sFRP2. Other partners have been reported for PCPE-1, including β2-microglobulin [93], laminin-111, endostatin (a fragment of collagen XVIII), collagen IV and collagen VI [87] but the functional implications of these interactions still require further investigation.

Finally, it was recently shown, in fibroblasts, that the production, secretion and cell surface localization of PCPE-1 are boosted by ascorbic acid (vitamin C) [94], an essential co-factor for correct hydroxylation, folding and secretion of procollagens. In the absence of evidence for the presence of hydroxyprolines in PCPE-1, the authors suggest that the effect of ascorbic acid on PCPE-1 is indirect and could derive from its enhanced co-secretion with procollagens. Such an early association would facilitate efficient subsequent extracellular procollagen maturation. Whether PCPE-1 could also act as a procollagen chaperone is more debatable, as an early report showing that PCPE-1 binds to the triple helix [45] could not be confirmed [78] and it is not known if its sole binding to the CP of procollagens [78] could have a significant stabilizing effect. Also, the presence of PCPE-1 or CUB1CUB2 during fibrillogenesis does not seem to affect the kinetics of fibril formation nor the type of fibrils that are formed [46,75], indicating that the role of the enhancer probably ends after procollagen processing.

Involvement of PCPE-1 in tissue repair and fibrosis

As described above, fibrosis is mainly defined by the aberrant tissue accumulation of extracellular matrix components [5,95,96], among which fibrillar collagens I and III are the most abundant. It can be triggered by a great diversity of acute or chronic injuries, ranging from apparently benign skin wounds, leading to hypertrophic scars after a few weeks, to chronic hypertension causing myocardial fibrosis and heart failure after several years of silent remodelling. If fibrosis can be a beneficial reparative component of wound healing and tissue repair in the initial phase, chronic and uncontrolled fibrosis becomes highly pathogenic and impairs normal organ function [97]. Main players in fibrosis are myofibroblasts which derive from different cell types depending on the organ but share similar features of high contractility and ability to secrete large amounts of ECM proteins [98,99]. These cells are required to allow tissue repair but due to persistent activating mechanical and biochemical factors, they fail to leave the injured area, survive beyond the normal phase of tissue healing and continue to synthesize ECM.

Fibrosis principally refers to excessive ECM accumulation in internal organs such as heart, lung, kidney or liver. However, in tissues where the ECM content is already high in normal conditions such as skin and cornea, fibrosis can also reflect the abnormal organization of the ECM network with no or a limited increase in total ECM content. As a specific and potent regulator of collagen fibrillogenesis, PCPE-1 has early been considered as a potential contributor to fibrotic disorders [100–102]. However, it is only in recent years that the accumulation of data from various types of patient samples and animal models has made it evident that there is a strong link between fibrosis and PCPE-1 overexpression. These results are described below and show that up-regulation of PCPE-1 can be observed in all major organs known to develop fibrosis.

Cardiac fibrosis

Cardiac fibrosis is associated with most cardiovascular diseases (CVDs) and is one of the main factors that predispose to heart failure [103,104]. In the heart, myocardial stiffness leads to mechanical, electrical and vasomotor dysfunctions [105] which are typical features of heart failure. Myocardial infarction (MI), hypertension, coronary artery disease and valvular lesions are examples of CVD that trigger a tissue response to injury and often lead to aberrant tissue remodelling with excessive accumulation of ECM. While in some CVDs such as MI, damaged cells are replaced by ECM and a scar is formed through a mechanism often referred to as replacement (reparative) fibrosis, in other conditions affecting the heart such as dilated cardiomyopathy or hypertension, it is more a reactive perivascular and interstitial fibrosis which develops, i.e. the diffuse accumulation of collagen between myocytes [103,106]. Although the pathophysiological mechanisms leading to fibrosis (replacement or reactive) may differ, common

effectors are involved. These include fibroblasts and myofibroblasts, components of the renin-angiotensin-aldosterone system and the transforming growth factor-β1 (TGF-β1) [96,104,107].

In this context, it is interesting to note that treatment of adult rat heart fibroblasts with 2.5 ng/ml TGF-β1 was shown to increase the amount of PCPE-1 in culture medium by a factor around 2 [102]. In the same study, *Pcolce* mRNA and secretion of PCPE-1 protein were found to be modestly but significantly increased (around 1.5-fold) in the presence of 10 nM aldosterone. Also, spironolactone, an aldosterone receptor antagonist, restored the basal level of *Pcolce* mRNA which was upregulated 3-fold in a rat model of myocardial infarction after coronary artery ligation [100]. Normalization in the presence of spironolactone was also observed at the protein level, confirming that aldosterone directly stimulates PCPE-1 expression. Following this seminal study establishing that PCPE-1 expression is induced after MI, this finding was confirmed in other reports [108,109]. Atorvastatin, a cholesterol-lowering agent among the most prescribed drugs in the world, also proved its efficacy in counteracting the increased expression of PCPE-1 in a rat model of MI [109]. Similarly, rosuvastatin reduced myocardial fibrosis and *Pcolce* mRNA in a mouse model of metabolic syndrome induced by deficiency of leptin and LDL receptor [110].

Pressure overload secondary to chronic hypertension is another well-described cause of cardiac fibrosis. It can be induced in animal models by a continuous subcutaneous infusion of angiotensin II, a potent vasoconstrictor and upstream regulator of aldosterone production. After the first reports showing that PCPE-1 is more abundant in mouse heart after angiotensin II treatment [111,112], the relationship between angiotensin II, cardiac fibrosis and PCPE-1 induction was recently confirmed in a more extensive study combining the effect of angiotensin II with a NO synthase inhibitor in rats [113]. In this model, PCPE-1 protein was increased 6-fold at the heart tissue level and to a lesser extent in the circulation. It was also co-localized with fibrotic areas in the myocardium. Notably, both in this study and in the model of MI described above, there was an excellent correlation between the PCPE-1 protein quantified in the heart by immunoblotting and the amount of collagen I measured through immunoblotting or Sirius Red staining ($r = 0.82$ and 0.92) [100,113].

Another leading cause of reactive fibrosis with chronic hypertension is aortic valve stenosis (AS), the most common valvular disease in Western countries. Of great interest to establish the link between this disease and PCPE-1 was the study by Beaumont and colleagues [114] who analysed myocardial samples from the interventricular septum in a cohort of 28 AS patients versus 10 control samples by western-blotting. Although the data are not shown in the manuscript, these authors report a marked increase in the PCPE-1 levels of AS patients (11-fold). When the same patients were stratified between severe and less severe AS, PCPE-1 was also more abundant in the most severe cases. Finally, PCPE-1 appeared to be well-correlated with the collagen volume fraction and to a lesser extent with TGF-β expression in the same samples. In agreement with the induction of PCPE-1 in the context of left ventricular pressure overload, *Pcolce* mRNA was also shown to be elevated in murine models of transverse aortic constriction [115] or ascending aortic banding leading to dilated cardiomyopathy [116].

More generally, PCPE-1 has also been used to assess myocardial ECM remodelling, most of the time together with collagens I and III and with the cross-linking enzyme lysyl oxidase, and its upregulation was for example demonstrated when MI in sedentary rats was compared to MI in exercised rats [108], after subtotal nephrectomy in rats [117] or after ischemia–reperfusion injury in mice [118] and pigs [119]. All these results demonstrate that PCPE-1 overexpression broadly overlaps collagen overexpression and suggest a central role of PCPE-1 in both replacement and interstitial fibrosis of the heart.

Liver fibrosis

The second most well-studied fibrotic condition in relation with PCPE-1 is hepatic fibrosis. In the liver, the main causes of fibrosis are chronic viral infections, drug or alcohol consumption and metabolic disorders such as NASH (nonalcoholic steatohepatitis) which can elicit chronic inflammation and lead to the activation of effector cells, mainly hepatic stellate cells (HSCs) [120,121]. In mouse NASH liver for example, the fibrogenic pathway was shown to be enriched with a more than 3-fold increase in *COL1A1*, *COL1A2*, *COL3A1* and *PCOLCE* transcripts [122,123]. Liver fibrosis often develops into cirrhosis with several life-threatening complications such as portal hypertension. In 1997, Ogata e*t al.* carried out the first experiments suggesting a link between liver fibrosis and elevated PCPE-1 expression. They showed that HSCs derived from cirrhotic rat livers, obtained after 7 weeks of CCl_4 intraperitoneal injections, had higher *Pcolce* RNA content than normal liver HSCs [101]. A similar observation was made in endothelial cells freshly isolated from cirrhotic rats [124]. These findings were confirmed directly on cirrhotic livers where RNA and protein were detected [101] whereas PCPE-1 remained undetectable in normal liver [34,37,101].

More recently, the expression of PCPE-1 was monitored over time in mice both during the onset of fibrosis, over the 6 weeks of CCl_4 treatment, and during liver recovery after the treatment was stopped [125]. This analysis revealed that the amounts of circulating and tissue PCPE-1 closely

reflected fibrosis severity with a 9-fold increase in tissue level quantified by western-blotting at the end of CCl_4 injections and a peak in PCPE-1 plasma concentration, about 60% higher than normal, at the same time point. Furthermore, PCPE-1 expression fell to basal levels at the end of the recovery period, showing the reversibility of the up-regulation upon termination of the pro-fibrotic stimulus. In another study in rats, comparing oral administration of CCl_4 or two other pro-fibrotic chemicals for a short period of 5 days with compounds known to cause non-fibrotic liver injury, *Pcolce* was found to be the most differentially expressed gene among a set of 67 selected fibrogenic genes [126]. This result suggested that its expression pattern was specific to the fibrotic phenotype and that its up-regulation could be detected early after the induction of the fibrogenic response. As in cardiac fibrosis, *Pcolce* is beginning to be used to evaluate the fibrotic status of the liver and the efficacy of anti-fibrotic compounds in various preclinical models [127,128].

Despite the rather consistent results obtained in animal models, conclusions from the few available studies dealing with patient samples are presently rather confusing. A first study comparing PCPE-1 plasma concentration in five healthy controls and five patients with liver fibrosis caused by hepatitis B or C infection showed that the mean level of circulating PCPE-1 was higher in the liver fibrosis group, although this remained below statistical significance due to the small size of the cohort [129]. In contrast, compared to healthy individuals, the plasma concentration of PCPE-1 was lower in a cohort of 126 patients diagnosed with chronic hepatitis B for at least 6 months, thus indicating a negative correlation with fibrosis stage [130]. In addition, there was a huge difference in measured PCPE-1 concentrations between the two studies (basal levels in control subjects around 300 ng/ml in [129], in agreement with most other measurements of PCPE-1 plasma concentration [66,131], versus 4.5 ng/ml in [130]) and this questions the reliability of some of the commercial ELISA kits used to detect PCPE-1.

Pulmonary fibrosis

Fibrosis is also a characteristic of many respiratory diseases induced by environmental pollutants, infections, including tuberculosis [132] and COVID-19 [133], immune diseases or certain medications. Another common type of lung fibrosis is idiopathic pulmonary fibrosis (IPF), a chronic and progressive interstitial lung disease of unknown origin which is often fatal with a survival time of less than 5 years after diagnosis [134]. It is characterized by alterations of cellular composition and ECM accumulation with patchy scarring that cause irreversible damage to lung structure and function leading to respiratory failure [135].

In recent years, the matrisome of normal and diseased lung has been extensively described [136,137] and PCPE-1 was found to be a component of normal lung. The most complete set of data concerning its expression in lung fibrosis derives from the proteomic study performed by Schiller and colleagues in a classical mouse model of lung fibrosis induced by bleomycin [136]. The first interesting finding was that the solubility profile of PCPE-1 was significantly altered upon bleomycin treatment, with a global increase in protein solubility, suggesting a loss of its potential binding partners [87] in the fibrotic lung ECM. Despite this increased solubility, PCPE-1 was not detected in the bronchoalveolar lavage fluid (BALF) proteome of these mice. In terms of kinetics, the abundance of PCPE-1 was shown to peak between days 3 and 14 after bleomycin administration, corresponding to the fibrogenic phase, and then to diminish during the recovery phase. The maximum fold-change (2.7) was somewhat lower than in the models of cardiac and liver fibrosis described above, a characteristic that was also observed in patients when freshly extracted IPF fibroblasts were compared to normal lung fibroblasts [138] or when IPF lung biopsies were compared to control lung biopsies at the RNA [139] or protein level [140]. This suggests that PCPE-1 might be less directly necessary to the progression of pulmonary fibrosis than in cardiac or liver diseases.

Kidney fibrosis

Chronic kidney disease (CKD) is mainly a consequence of diabetes, hypertension or glomerulonephritis and is highly prevalent in the adult population. A common pathway in CKD is fibrosis which can ultimately lead to end-stage kidney failure with ECM accumulating in the glomeruli (glomerulosclerosis) and/or in the tubulo-interstitial space (renal interstitial fibrosis) [141]. PCPE-1 expression in the kidney is low in normal conditions [34,37] but seems to increase with the progression of CKD.

First, the *PCOLCE* gene was found to be located in close proximity to a single nucleotide polymorphism associated with the development of diabetic kidney disease and was included in a panel of 226 transcripts for which correlation with kidney function (quantified through the estimated glomerular filtration rate or eGFR) was analysed [142]. A moderate but significant inverse correlation was observed between *PCOLCE* expression and eGFR in the glomeruli, indicating increased expression of PCPE-1 when kidney function was decreased. In contrast, there was no correlation between *PCOLCE* transcript and kidney function in the tubules. Furthermore, the mean serum level of PCPE-1 measured by ELISA was 76% higher in a separate cohort of 131 CKD patients compared to 34 healthy controls [131]. Of note, the highest recorded serum concentration was 325 ng/ml in

the control group and 840 ng/ml in the patient group albeit that large variations were observed in both groups. Finally, circulating PCPE-1 was also inversely correlated with glomerular filtration rate and positively correlated with body mass index. What remains to be established to consolidate present data is a direct link between PCPE-1 levels and histological evidence of kidney fibrosis.

Ligament and muscle fibrosis

Like all the organs described above, ligaments and muscles can be affected by injury and activate a wound healing response. In some cases, healing will result in scar formation and fibrosis. Thus, the semi-quantitative mRNA analysis of some targets related to collagen fibrillogenesis in a rabbit model of ligament injury and scarring showed that the *PCOLCE* transcript had the highest level of up-regulation after 3 weeks when compared to *BMP1*, *LOX* and *TGFB1* transcripts [143]. It then went down but was still somewhat higher than normal levels after 14 weeks, a behaviour that differed substantially from the progression observed during normal ligament development and could explain why ligaments often fail to regenerate.

In the field of inherited muscle diseases, Duchenne muscular dystrophy (DMD) is known to lead to skeletal muscle degeneration and necrosis, inflammation and fibrotic remodelling [144]. Interestingly, PCPE-1 expression has been well characterized in a widely-used model of DMD, the *mdx* mouse which has a spontaneous mutation in the dystrophin gene and develops a mild form of the disease. In the fibrotic diaphragms of the *mdx* mice collected at 4 and 8.5 months of age, there was an overall increase in PCPE-1, detected by immunoblotting and immunofluorescence, which was not quantified but seemed to follow the marked increase in collagen I [125]. Notably and similarly to the observations made in models of cardiac and liver fibrosis [100,113,125], PCPE-1 and collagen I were also co-localized in diaphragm tissue. Finally, this study showed that the elevation of PCPE-1 levels in *mdx* mouse plasma was comparable to that observed for the N-propeptide of collagen III (PIIINP), an established marker of fibrosis [145].

Another group analysed the link between PCPE-1 and muscle fibrosis in OPMD (oculopharyngeal muscular dystrophy), a genetic disorder first affecting muscles of the eyelid, throat and proximal limbs due to a polyalanine expansion at the N-terminus of the PABPN1 (poly(A)-binding protein nuclear 1) protein. The results point to a completely novel mechanism by which mutant PABPN1 entraps PCPE-1 in nuclear aggregates leading to its exhaustion from the ECM [146]. ECM depletion and nuclear co-localization with PABPN1 aggregates can be observed in symptomatic OMPD patient muscle biopsies and in a mouse model of the disease. These observations are accompanied by a significant reduction in *PCOLCE/Pcolce* mRNA in symptomatic but not pre-symptomatic OPMD patients/mice when compared to age-matched controls. Importantly, the mechanism of PCPE-1 sequestration in the nucleus seems to be shared with another inherited muscle disease, myotonic dystrophy type 1, but not with DMD and facioscapulohumeral muscular dystrophy [146]. This study reveals that disease-specific mechanisms can also play a role and challenge the general trend of PCPE-1 overexpression in fibrosis-related conditions.

Corneal scarring

The cornea can also be exposed to different types of injuries such as refractive surgery, physical traumas, burns or infections. Due to the very specific organization of the collagen network required to maintain corneal transparency [7], defects in corneal wound healing lead to persistent non-transparent scars associated with a loss of visual acuity [147]. Soon after its discovery, PCPE-1 was shown to be highly expressed in the cornea [37] and, more recently, Malecaze *et al.* demonstrated that it was further increased at the RNA level between days 14 and 28 in a murine model of full-thickness excision leading to scarring, both in the corneal stroma and epithelium [53]. The protein detected by immunostaining also seemed substantially more abundant in the stroma, a trend that was qualitatively confirmed in four human corneal scars of various etiologies [53]. A subsequent study extended these results to other murine models of corneal injuries [54]. Five days after an alkali burn, PCPE-1 seemed rather decreased in the wound area but was highly concentrated at the epithelial-stromal junction juxtaposed to the burn zone. Another model of epithelial abrasion led to a stronger immunofluorescence signal for PCPE-1 in the stroma at day 5 but, intriguingly, this was preceded by the transient accumulation of the protein just beneath the basement membrane between days 1 and 3. In these various models, the lack of *Pcolce* did not change the grade of the opacity but delayed epithelial closure and neutrophil recruitment and promoted angiogenesis [54]. The mechanisms underlying these observations are presently not understood.

Skin scarring

Hypertrophic scars (HTSs) and keloids are the most common manifestations of skin fibrosis. They occur after a wound healing response has been triggered, when activating signals fail to disappear. Whereas normal scars usually appear as fine and flat lines and regain up to 80% of original skin tensile strength, HTSs are raised, erythematous, sometimes pruritic and painful, and can cause contractures affecting mobility. However, they remain restricted to injury area in

contrast to keloid scars which can grow outside injury boundaries and evolve into benign dermal tumors that usually recur after excision [148,149]. Like in other fibrotic disorders, TGF-β signaling is largely demonstrated to be a critical regulator of skin scarring [150,151]. In this context, it is interesting to note that the addition of 10 ng/ml TGF-β1 to normal human dermal fibroblasts had no effect on PCPE-1 synthesis ([152] and our unpublished results), in contrast to the positive effect described for heart fibroblasts [102], suggesting that the effect of the growth factor on fibroblasts depends on tissue origin. To the best of our knowledge, the effect of TGF-β1 on the synthesis of PCPE-1 by HTS and keloid fibroblasts has never been directly tested but there are some data suggesting that PCPE-1 could still be an important driver of collagen aberrant accumulation in skin scars. First, secreted PCPE-1 protein seems to be significantly more abundant (more than ten-fold) in cultures of HTS fibroblasts, as assessed by immunoblotting and compared to normal fibroblasts [153]. Second, *PCOLCE* mRNA was up-regulated in skin biopsies from HTSs and keloids [154,155]. Finally, treatment of fibroblasts extracted from the same type of skin scars by rapamycin, an mTOR inhibitor known to hinder fibroblast proliferation and ECM/collagen synthesis, decreased *PCOLCE* transcription between 58 and 69% in normal, HTS and keloid fibroblasts [155].

PCPE-1 as a biomarker of fibrosis

The studies described above suggest that PCPE-1 could be an excellent tissue marker to reveal fibrotic lesions. Tissue biopsies remain widely used for diagnosis and disease monitoring in the field of fibrosis-related diseases despite their invasiveness and possible sampling errors. However, there are already established collagen-specific histological methods to detect fibrosis in tissue sections and it is unlikely that PCPE-1 immunostaining would bring any added-value. More promising therefore is the development of methods allowing its detection through non-invasive approaches such as quantitation in easily-accessible body fluids. The search for such new biomarkers in the field of fibrosis is extremely active, either because reliable and sensitive markers are presently missing or because they are not specific or insufficient. In this context, PCPE-1 has a strong potential even if several steps will have to be validated before its clinical use can be considered.

What is presently well established is that PCPE-1 is found in human serum and plasma and can be easily detected and quantified using several techniques such as immunoblotting, ELISA and mass spectrometry [66,113,129,131,156]. According to present literature and public databases, PCPE-1 is also found in cerebrospinal fluid [157–160] but almost undetectable in saliva, urine or broncho-alveolar fluid. Even though the most comprehensive studies establishing the relationship between the severity of tissue fibrosis and circulating levels of PCPE-1 were performed in murine models [113,125,126], measuring PCPE-1 in human plasma has also revealed elevated concentrations in diseased individuals with a strong suspicion of liver or kidney fibrosis [129,131]. Moreover, there are some good hints that PCPE-1 monitoring allows the early detection of fibrogenic/pre-fibrotic stages and is closely correlated with fibrosis severity [125,126,136]. The most urgent need is hence to strengthen patient studies by the orthogonal evaluation of tissue fibrosis and to extend them to larger cohorts or new fibrotic disorders.

While an ideal biomarker should be specific to the targeted disease, it is likely that several concomitant conditions occurring in parallel to fibrosis will affect PCPE-1 blood levels. This is the case for closely-related collagen metabolites (collagen I and III propeptides or telopeptides) that show variations associated with age, sex, bone remodelling or pregnancy [161–163]. Whether PCPE-1 circulating levels display the same variations remains to be analysed but it is already known that bone disorders can affect its concentration and glycosylation pattern in human serum [66]. However, it should be noted that truly specific biomarkers of fibrosis are very rare and combinations of biomarkers are often considered to be more reliable [164–167]. In summary, PCPE-1 represents a promising biomarker of fibrosis, alone or in combination with other biomarkers, but a more extensive evaluation in fibrotic patient samples is required before its detection can be implemented in clinical practice.

PCPE-1 as a therapeutic target to prevent fibrosis

As broadly exemplified above, PCPE-1 is found in larger amounts in fibrotic tissues than in normal tissues, both at the RNA and protein levels, with very few exceptions. This does not show whether PCPE-1 overexpression is a causative factor of fibrosis or just an indirect consequence of the fibrotic environment. However, current knowledge about its direct contribution to the last step of procollagen maturation just preceding fibril formation, its specificity for collagen-related processes, its co-localization with collagen in several types of fibrotic scars and its mechanism of action requiring a 1:1 ratio with procollagen for maximal enhancement, all point at a direct and prominent contribution to aberrant collagen deposition in fibrosis. Curiously, the effect of the absence of PCPE-1 has only been studied in models of corneal scarring and no clear positive effect on collagen abundance or corneal transparency could be evidenced [54]. Due to the high content of collagen in cornea, this might not be the best model to establish that PCPE-1 depletion or inhibition can significantly affect further collagen integration in ECM and other models dealing

with less collagen-rich organs might be more informative. Thus, what is critically missing at this point is the combination of a model of cardiac, kidney or liver fibrosis with PCPE-1 overexpression, deficiency or inhibition to demonstrate that the modulation of PCPE-1 activity can significantly impact the collagen volume fraction and the function of these organs.

Nevertheless, we can already speculate that a great advantage of targeting PCPE-1 in reparative fibrosis processes would be that BTPs will remain active at their basal level and can ensure that a minimum amount of properly matured collagen fibrils continue to be synthesized to repair the injured tissue. Indeed, the complete blockade of collagen deposition is known to have detrimental effects on normal and injured adult tissues, as demonstrated by the analysis of the conditional double *Bmp1*/*Tll1* knock-out mice [168–170]. Very illustrative is the fact that BTP ablation in young adult mice leads to a progressive reduction in the thickness of skin dermis and to never-healing wounds [168].

Another obvious strong point of PCPE-1 as a target, especially in contrast with PCPs and PNPs, is its specificity for procollagen maturation. As described above, it does not seem to affect the processing of any of the BTP substrates beside fibrillar procollagens [44,51,52] and the potential TIMP-like activity of its NTR domain was not confirmed [73]. The procollagen specificity also implies that other pro-fibrogenic BTP substrates such as pro-lysyl oxidases or those involved in the regulation of TGF-β signalling [22,48] are not under the direct regulation of PCPE-1. However, the role of matrix stiffening in the amplification of fibrogenesis was clearly demonstrated in recent years [171,172] and we can speculate that PCPE-1 also contributes to fibrosis by increasing collagen fibril density and therefore tissue mechanical strains.

At this point, it is important to mention that PCPE-1 specificity has been challenged by some reports suggesting possible roles of the protein in angiogenesis [54,87,173], cell proliferation [174,175] or RNA stabilization [176]. Among these, the role in angiogenesis is the most-well supported and should be considered seriously. First, PCPE-1 was found to belong to a group of five ECM proteins synthesized by fibroblasts (together with collagen I) required to promote endothelial cell lumen formation [173]. Second, PCPE-1 can strongly bind to several anti-angiogenic factors such as endostatin and thrombospondin-1 and displays anti-angiogenic properties itself, both in *in vitro* assays [54,87] and in an *in vivo* model of corneal wound healing induced by alkali treatment [54]. There is presently no clear mechanism to explain these observations and it remains possible that the control of angiogenesis by PCPE-1 is an indirect consequence of its role in collagen maturation.

In terms of drug development, we know that PCPE-1 forms a tight complex with procollagens I and III [33,44,77]. Even if protein–protein interactions are known to be difficult targets, it should be possible to develop molecules capable of binding either the enhancer or the procollagen motifs involved in the interaction with the latter. Both can be considered and should lead to efficient slowdown of procollagen maturation but with slightly different consequences. A pharmacological molecule targeting PCPE-1 would block its interaction with the CPs of all three major fibrillar collagens (I, II and III) while targeting the CP of one procollagen would, in principle, diminish the maturation of this specific molecule without affecting the maturation of other procollagens. Given the previously described interdependence between fibrillar collagens [13,14], the latter approach would require determination of how the collagen network can cope with the delayed incorporation of one collagen type. However, considering the high level of homology between the CPs of procollagens I, II and III (46% identity), it might actually be easier to develop a pan-CP inhibitory molecule and, in this case, both strategies should have similar outcomes.

Other related questions remain regarding the fate of unprocessed procollagens in the context of a fibrosis-related rapid procollagen synthesis combined with a decrease in maturation efficiency. In the preferred scenario, they would be rapidly degraded by MMPs or other ECM-degrading proteases released by fibroblasts and inflammatory cells. Overall collagen degradation, which is thought to be insufficient in the context of fibrosis, could here benefit from collagen intermediates being more easily accessible for proteolytic degradation than fibril-embedded and highly cross-linked collagen monomers. In another scenario, unprocessed procollagens would persist in the tissue and could potentially trigger signalling in surrounding cells, including in those involved in the resolution of fibrosis. Along this line, addition of CPI to cultures of human lung fibroblasts was early found to induce a dose-dependent decrease in collagen synthesis [177] and, although this result has never been confirmed or explained, it suggests that released C-propeptides could exert a negative transcriptional feedback on collagen synthesis. Whether the same type of signalling is also induced when CPs are retained in procollagens or whether the lack of CP sensing by cells would rather increase collagen synthesis remains to be analysed. In addition to the previous outcomes, it remains possible that unprocessed or partially-processed procollagen molecules are aberrantly incorporated into the few newly formed fibrils. However, if C-propeptide retention in collagen fibrils was previously observed in the $Bmp1^{-/-}/Tll1^{-/-}$ mouse embryonic cell layers, this was not the case in the $Pcolce^{-/-}$ cell layers [46], giving a good indication

Table 1 Pros and cons of targeting PCPE-1 activity to prevent collagen accumulation in fibrosis.

Pros	Cons
PCPE-1 activity significantly increases collagen fibrillogenesis Basal collagen assembly remains possible to allow tissue repair	Delaying procollagen processing could not be sufficient to observe a significant reduction of tissue fibrosis
Absence of PCPE-1 does not lead to C-propeptide retention in collagen fibrils	Consequences of the possible accumulation of unprocessed procollagen molecules in fibrosis models are unknown
PCPE-1 is highly specific to collagen biosynthesis and increased PCPE-1 expression is found at sites of high collagen deposition	PCPE-1 unexplained role in angiogenesis remains to be explored
Structural data are available to facilitate drug development	Protein-protein interactions are difficult to target with small-molecule inhibitors
Anti-PCPE strategies can be applied to multiple potential indications in fibrosis	Deleterious effects of long-term PCPE-1 inhibition on tissue homeostasis or in case of co-morbidity (e.g. wounds, bone fractures) cannot be excluded

that collagen processing intermediates do not become abnormally integrated in fibrils when PCPE-1 activity is missing.

As summarized in Table 1, PCPE-1 has many clear advantages as an anti-fibrotic target but several questions still need to be addressed to confirm its potential. Especially, future work in the field should aim at clarifying the overall effect of modulating PCPE-1 expression or activity on collagen abundance and organization.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no competing financial interests.

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Abbreviations:
ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; AS, aortic valve stenosis; BMP, bone morphogenetic protein; CVD, cardiovascular disease; CKD, chronic kidney disease; CP, C-propeptide; CUB, complement, Uegf, BMP-1; DMD, Duchenne muscular dystrophy; ECM, extracellular matrix; EGF, epidermal growth factor; eGFR, estimated glomerular filtration rate; ELISA, enzyme-linked immunosorbent assay; HDL, high-density lipoprotein; HSC, hepatic stellate cell; HTS, hypertrophic scar; IPF, idiopathic pulmonary fibrosis; LDL, low-density lipoprotein; MI, myocardial infarction; MMP, matrix metalloproteinase; mTLD, mammalian tolloid; mTLL, mammalian tolloid-like; NASH, nonalcoholic steatohepatitis; NTR, netrin; PABPN1, poly(A)-binding protein nuclear 1; OPMD, oculopharyngeal muscular dystrophy; PCP, procollagen C-proteinase; PCPE, procollagen C-proteinase enhancer; PNP, procollagen N-proteinase; SPC, subtilisin proprotein convertase; TIMP, tissue inhibitor of metalloproteinases; TGF-β, transforming growth-factor β; TSPN, thrombospondin-like N-terminal

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