



What does procollagen C-endopeptidase enhancer protein 2 have to do with HDL-cholesteryl ester uptake? Or how I learned to stop worrying and love reverse cholesterol transport?

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Purpose of review

The purpose of this study is to provide an update on the role HDL apolipoprotein A-I plays in reducing the risk of cardiovascular disease (CVD) and how it relates to reverse cholesterol transport (RCT).

Recent findings

Despite numerous studies showing that plasma HDL cholesterol concentrations are correlated with a reduced risk of CVD, pharmacologic elevation of HDL has not shown any beneficial effects to date. In contrast, studies correlating the measure of an individual's plasma cholesterol efflux capacity show greater promise as a tool for assessing CVD risk. Although ATP-binding cassette transporter 1-mediated lipidation of apoA-I is considered the principal source of plasma HDL, it represents only one side of the RCT pathway. Equally important is the second half of the RCT pathway in which the liver scavenger receptor class B1 selectively removes HDL cholesteryl esters for excretion. The combined action of the two enzyme systems is reflected in the overall steady-state concentration of plasma HDL cholesterol. For example, reduced ATP-binding cassette transporter 1-mediated production of nascent HDL lowers plasma HDL concentration, just as an increase in cholesteryl ester uptake by scavenger receptor class B1 reduces HDL levels. Thus, the complexity of intravascular HDL metabolism suggests that steady-state plasma HDL concentrations do not provide adequate information regarding an individual's HDL quality or function. Herein, we describe a new player, procollagen C-endopeptidase enhancer 2, which shows atheroprotective function and influences both sides of RCT by enhancing production and catabolism of HDL cholesteryl esters.

Summary

The discovery of a new molecule, procollagen C-endopeptidase enhancer 2, implicated in the regulation of HDL cholesteryl ester concentrations suggests that the extracellular matrix and the proteins that regulate its function represent a new and as yet unexplored realm of HDL cholesterol metabolism.

Keywords

cholesteryl esters, extracellular matrix, HDL, procollagen C-endopeptidase enhancer 2, scavenger receptor class B1

INTRODUCTION

For over 40 years, it has been assumed that higher plasma levels of HDL cholesterol are associated with a reduced risk for cardiovascular disease (CVD) [1]. This belief is based on HDL's repeatedly documented role in reverse cholesterol transport (RCT), although detailed mechanisms explaining how HDL protects against cholesterol accumulation in the artery wall remain controversial. Compounding this, recent attempts to pharmacologically increase HDL levels have not led to increased benefit [2], suggesting that HDL concentration does not accurately reflect HDL function. Leading the focus

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KEY POINTS

- HDL is the intermediary between several proteins that are essential components of the RCT pathway responsible for whole body cholesterol homeostasis. The 'meaning' of reduced CVD risk relative to plasma HDL concentration is being re-evaluated with regard to the mechanism(s) that reduces risk.
- It is the metabolic interaction between all RCT components that contributes to reducing the risk for CVD. PCPE2 is an essential component of RCT that contributes to its function, however, as the newest member of what has been heretofore regarded as a well understood pathway, highlights what is not known about the mechanism of RCT.
- CUB domains like those found in PCPE2 bind proteins such as apoA-I. The NTR tail anchors PCPE2 to glycosaminoglycans of the ECM and helps position it to deliver CUB-bound apoA-I to SR-B1.

on HDL function, two studies show that the efficiency of cholesterol efflux to LDL-depleted plasma [3] correlates with reduced risk for CVD in humans [4,5²²]. In these studies, cholesterol efflux was mostly mediated by the ATP-binding cassette transporter A1 (ABCA1) and correlated with the concentration of lipid-poor pre β HDL particles [3]. How this rare cholesterol-depleted HDL particle is continuously generated from mature HDL at the artery wall is not entirely understood [6,7,8²³,9,10], leaving this and other important questions unanswered.

BODY

HDL concentration, reverse cholesterol transport and atherosclerosis

Several thorough reviews of cholesterol efflux, RCT and atherosclerosis have been published over the last several years [11–13]. In this review, we will emphasize recent studies into the role of the newly discovered player, procollagen C-endopeptidase enhancer 2 (PCPE2), which resides in the extracellular matrix (ECM) and plays a role in both RCT and atherosclerosis. Given that steady-state plasma HDL concentrations do not provide sufficient information on either cholesterol efflux capacity or overall HDL function, it follows that other factors that impart atheroprotective function should be examined whether they reside on the HDL particle or enable optimal processing of HDL's cholesterol cargo.

Accessory proteins, cholesterol efflux and nascent HDL production

Several reports suggest that accessory proteins located on the plasma membrane assist in ABCA1-

mediated assembly of nascent HDL (nHDL) [14–19], where nHDL represents the product(s) of lipid-poor apoA-I's interaction with ABCA1 [20]. Many years ago, it was discovered that newly synthesized apoA-I contained a hexapeptide pro-segment as well as a signal peptide (prepeptide). The presegment was removed at the time of synthesis, but the pro-segment was removed during secretion [21–23]. Over 9 years since the pro-apoA-I sequence was described, many questions about this process have been answered [24–29]. It is now known that the removal of the pro-peptide stimulates the rate of apoA-I intracellular translocation and that bone morphogenetic protein 1 (BMP1) cleaves the apoA-I propeptide [17,24] at the surface of the cell affecting the rate of lipidation *in vitro*, and thus the production rate of nHDL.

The enzyme that cleaves apoA-I's propeptide, BMP1, is an astacin metalloprotease with diverse substrates including ECM proteins and antagonists of the transforming growth factor β (TGF β) superfamily [30,31]. It is best known for cleaving C-terminal propeptides from procollagen precursors. This cleavage is essential for self-assembly of mature collagen monomers into fibrils within the ECM [32,33]. Also involved in this process is the enhancer protein, procollagen proteinase enhancer protein 1 or PCPE1 (*PCOLCE* gene). This protein stimulates the procollagen C-proteinase activity of BMP1 [34]. PCPE2 (*PCOLCE2* gene) is related to PCPE1 sharing 43% amino acid identity with similar domain structure, but having markedly different glycosylation than PCPE1, and assisting BMP1 in modifying collagen [35,36]. However, the tissue distribution of the PCPEs is somewhat different with PCPE2 more highly expressed in heart, aorta and adipose, while PCPE1 shows a wider expression pattern. Both are glycoproteins having two Complement C1r/C1s, Uegf, Bmp1 (CUB) domains (Complement C1r/C1s, Uegf, Bmp1) separated by a short linker region, with each domain containing a β -sandwich fold that mediates a variety of protein–protein interactions [37–41]. The CUB domains have a homologous Ca²⁺-binding site that mediates ionic interactions between protein partners [38], similar to that described for the LDL receptor family [42,43]. PCPE2 also has a netrin-like (NTR) domain [32,33,44,45] that binds cell surface heparan sulphate proteoglycans (HSPGs) anchoring it to the ECM. Once believed to inhibit BMP1, the NTR region is now known to stimulate enhancer activity in the presence of HSPG [33]. From these studies, it appears that PCPE2 binds to HSPG in the ECM and then through one or both of its CUB domains coordinates the enzymatic activity of BMP1 whether it be that of procollagen or the six amino acids from

proApoA-I. In addition to these functions, several recent reports link PCPE2 as a contributor to disorders characterized by fibrosis of the pancreas [46], as well as to TGF- β 1 stimulation of human amniotic fluid derived mesenchymal cells [47], arachidonic acid abundance in red blood cells [48[¶]] and collagen accumulation associated with chronic pressure overload in mouse heart [49].

Procollagen C-endopeptidase protein 2, nascent HDL formation and cholesterol efflux

To explore the relationship between PCPE2 and nHDL formation, Zhu *et al.* [50] investigated the binding interactions between PCPE2, BMP1 and proapoA-I using surface plasmon resonance (SPR) and selective immunoprecipitation. Their studies showed that PCPE2 forms a high-affinity complex with apoA-I and BMP1 and more importantly that PCPE2 serves as a cofactor reducing the K_d . This study provided strong biophysical support for the notion that PCPE2 plays a role in propeptide cleavage. In addition, clinical studies show significant correlations between PCPE2 polymorphisms and HDL [51], suggesting that PCPE2 may have other significant physiologic consequences from its interactions with apoA-I.

PCPE2 was originally a candidate gene for glaucoma [52], but now best known for its relationship with HDL concentrations in three independent population cohorts [50,51,53]. Associations in these studies were modest, but led investigators to carry out studies in PCPE2^{-/-} mice [54], which revealed elevated concentrations of enlarged HDL particles. In addition, Francone *et al.* [53] showed that apo B depleted plasma from PCPE2^{-/-} mice was defective in its ability to mediate in-vitro cholesterol efflux via ABCA1 and when combined with their enlarged HDL led to the hypothesis that HDL particles were dysfunctional. These seminal studies provided the first critical proof-of-concept data showing that PCPE2 plays a pivotal role in HDL metabolism. More recent support for the role of PCPE2 in HDL metabolism comes from genome-wide association studies showing that PCPE2 is a significant modulator of hepatic apoA-I secretion [55].

Procollagen C-endopeptidase protein 2, atherosclerosis and HDL cholesteryl ester catabolism

Intrigued by the paradoxical observations of Francone *et al.* [53], Pollard *et al.* [56] investigated whether the higher concentration of enlarged HDL associated with the loss of PCPE2 was atherogenic or atheroprotective. To do this, they crossed

PCPE2^{-/-} with LDLr^{-/-} mice to obtain LDLr^{-/-} PCPE2^{-/-} mice. After feeding LDLr^{-/-}, PCPE2^{-/-} and LDLr^{-/-} mice a Western diet for 12 weeks, the aortic root was examined for neutral lipid and immune cell content. Interestingly, LDLr^{-/-} PCPE2^{-/-} mice had a greater extent of aortic root neutral lipid and CD68⁺ accumulation than did LDLr^{-/-} mice. Furthermore, these mice showed a similar extent of neutral lipid staining and immune cell accumulation as LDLr^{-/-} ApoA-I^{-/-} mice that possess no HDL apoA-I. Taken together, this suggested that in the absence of PCPE2, the increased levels of enlarged HDL were completely dysfunctional, showing for the first time that PCPE2, an extracellular matrix associated protein, confers atheroprotective function to HDL particles *in vivo*.

To explore the basis for the increased concentrations of enlarged HDL, catabolic studies were conducted using HDL isolated from both diet-fed LDLr^{-/-} PCPE2^{-/-} and LDLr^{-/-} mice. The plasma decay of ¹²⁵I apoA-I labelled HDL was monitored as a function of time and found to be delayed in LDLr^{-/-} PCPE2^{-/-} compared with LDLr^{-/-} mice, regardless of the source of HDL particles. These studies suggested that PCPE2 was acting at the level of the tissues/cells and not by virtue of the HDL particle or its cargo. To examine the effect of reduced HDL catabolism on RCT, [³H]-cholesterol loaded J774 cells were intraperitoneal injection injected into mice and their appearance in plasma and faeces monitored. Mice lacking PCPE2^{-/-} showed significant attenuation of RCT [56], suggesting that liver scavenger receptor class B1 (SR-B1) may be impaired.

In mice, increased HDL size and concentration is a hallmark of SR-B1 deficiency [57,58]. Hepatic-specific SR-B1 deficient mice show greater aortic lesion formation and reduced macrophage to liver RCT [59], despite higher HDL concentrations. As reported in genetically modified mice, humans with genetic variants in SR-B1 show a reduced capacity to efflux cholesteryl ester, which leads to greater HDL plasma concentrations and a greater risk for CVD [60,61^{¶¶},62^{¶¶},63]. The PCPE2-deficient mice showed a macrophage to faecal RCT rate significantly lower than control LDLr^{-/-} mice and similar to that reported for SR-B1 knockout mice. Interestingly, this lower rate occurred despite a two-fold higher level of SR-B1 protein in the livers of LDLr^{-/-}, PCPE2^{-/-} mice [56], prompting a closer look at how PCPE2 might influence SR-B1 function.

To imagine how PCPE2 might influence SR-B1 function, an understanding of SR-B1 structure, is necessary. Details of SR-B1's conformation have been inferred by analogy to the structure of LIMP-2 [64], although a much needed NMR structure nears completion [65[¶]]. What is known, SR-B1's N- and

C-terminal domains span the plasma membrane [66], while the heavily N-glycosylated central region loops out into the extracellular matrix wherein it interacts with HDL particles. The exact mechanism explaining how and where SR-B1 selectively removes HDL cholesteryl ester and in the process releasing lipid-poor apoA-I remains to be fully explained [67,68^{*}]. However, mounting evidence suggests that SR-B1 achieves selective cholesteryl ester uptake following oligomerization [69,70], while the cytoplasmic C-terminal region of SR-B1 bound to Na(+)/H(+) exchange regulatory cofactor NHE-RF3 protein encoded by the PDZK1 gene, a four PDZ domain containing adaptor protein, confers localization [71].

To understand how PCPE2, an enhancer protein found in the ECM, might influence SR-B1 function, Pollard *et al.* [56] overexpressed PCPE2 in Chinese hamster ovary cells and measured a two-fold increase in the uptake of HDL ³H-cholesteryl oleyl ether. In these studies, SR-B1 protein levels were unchanged but a shift to a higher fluorescence intensity was noted, suggesting that the presence of PCPE2 on the cell surface may induce conformational shift(s) in the extracellular loop region of SR-B1 corresponding to amino acids 38–440 either through direct protein–protein interaction or by enhancing SR-B1 oligomerization on the membrane surface.

Model of PCPE2 influence on SR-B1 function and physiological implications

SR-B1 belongs to the scavenger receptor protein family of which there are at least eight classes [72]. Using accumulated information, it is believed that class B receptors form multimolecular complexes or signalosomes in which they mediate chaperone functions and ligand internalization, despite the absence of discernable signalling motifs. Several different approaches have shown that SR-B1 employs its PDZ-interaction domain and the C-terminal transmembrane domain for HDL-initiated signalling [73], which involves cholesterol sensing. Thus, the physiological implications of SR-B1 mediated HDL signalling have been implicated in a variety of systems related to the maintenance of haematopoietic stem cell [74^{*}] and lymphocyte cholesterol homeostasis [75], which could play an important role in the development of atherosclerosis. To better understand how an extracellular matrix localized collagen processing enhancer protein impacts SR-B1 function, a hypothetical model shown in Fig. 1 is described. PCPE2 binds to HSPGs, which are found in the extracellular matrix. One or both CUB domains found in PCPE2 bind to HDL apoA-I and assist in localizing or altering particle stability assisting in the movement of HDL cholesteryl ester to SR-B1 for selective uptake.

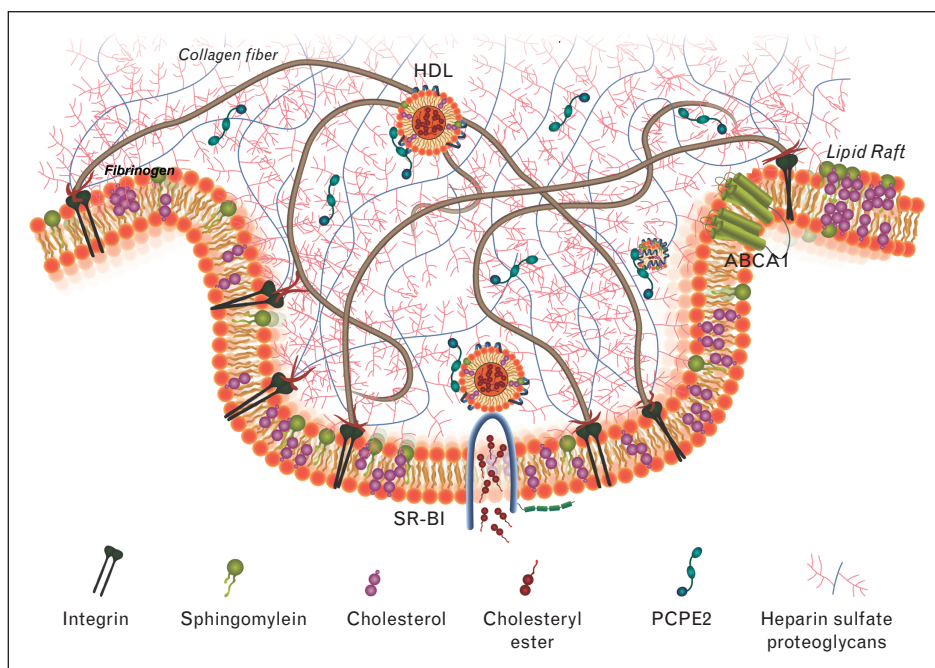


FIGURE 1. The role of procollagen C-endopeptidase protein 2 (PCPE2) in reverse cholesterol transport. PCPE2 binds to the extracellular matrix through heparin sulphate proteoglycans. One hypothesis of how PCPE2 stimulates scavenger receptor class B1 (SR-B1) cholesteryl ester uptake suggests that PCPE2 binds to plasma HDL thereby inducing conformational changes to the lipoprotein particle promoting the removal of HDL cholesteryl ester.

Lipid-poor apoA-I is then released by PCPE2 and it is either remodelled [76] or can bind ABCA1 and initiate the formation of nHDL particles.

CONCLUSION

The identification of a new player in the RCT pathway underscores the lack of understanding of HDL cholesteryl ester homeostasis in atherosclerosis. Because RCT and HDL play a pivotal role in cholesterol homeostasis and modulating atherosclerosis, increased attention to mechanistic details will yield new information for predicting risk and eventually show how to pharmacologically control the development of CVD.

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Conflicts of interest

M.S.T. has received honoraria from Eli Lilly & Co. and Merck & Co. M.J.T. and R.D.P. have no conflicts to declare.

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