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The emerging roles of PCSK9: more than a one trick pony

Kathryn J Moore[1] and **Ira J Goldberg**[2]

[1]Leon H Charney Division of Cardiology, Department of Medicine, New York University School of Medicine, New York, NY

[2]Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, New York University School of Medicine, New York, NY

Rare human genetic variations at the proprotein convertase subtilisin/kexin type 9 (PCSK9) locus revealed the role of this protein in regulating plasma levels of low density lipoprotein cholesterol (LDL-C) and its promise as a therapeutic target[1, 2]. PCSK9 promotes the internalization and degradation of the hepatic LDL receptor, thereby reducing the liver's ability to clear LDL from the circulation and causing LDL-C levels to rise[3, 4]. Monoclonal antibodies against PCSK9 that disrupt the interaction of this protein with the LDL receptor have been studied in numerous clinical trials, and these agents have been shown to safely reduce LDL-C by 50–60%[5]. Although large cardiovascular outcomes trials are still being conducted, two of these agents were recently approved by regulatory authorities on the basis of their LDL-C reductions alone, for the treatment of patients with familial hypercholesterolemia or those with established cardiovascular disease who require additional lipid lowering therapy despite maximally tolerated doses of statins.

The rapid development of a novel therapeutic can often outpace our understanding of the underlying biology, and this is becoming apparent in the case of PCSK9 function. Although most intensely studied for its interaction with the LDL receptor, it is now evident that PCSK9 can promote the degradation of other cell surface receptors, including close structural homologues of the LDL receptor (e.g. the VLDL-receptor and apolipoprotein E receptor)[6], the CD81 receptor for hepatitis C virus[7], the epithelial sodium channel (ENaC)[8], and beta-secretase 1 (BACE1)[9]. A study by Demers et al[10] in this issue of ATVB adds CD36 – a scavenger receptor with functions in fatty acid transport, lipoprotein uptake and innate immunity - to this growing list of PCSK9-interacting partners, and suggests that PCSK9 may have important metabolic roles beyond regulating plasma LDL-C.

CD36 is an archetypal multi-ligand scavenger receptor that binds native and modified lipoproteins, pathogen associated molecular patterns, and amyloidogenic peptides[11]. However, CD36 also binds long-chain fatty acids to facilitate their transport into cells and has important roles in muscle lipid utilization, adipose energy storage and hepatic triglyceride storage and secretion[11]. CD36 is found on the cell surface of a wide variety of cells and is characterized by two membrane spanning domains and a large, heavily N-

Address correspondence to: Kathryn J Moore, PhD, New York University School of Medicine, 522 First Avenue, Smilow 705, New York, NY 10016, tel: 212.263.6631 fax: 212.263.9115, kathryn.moore@nyumc.org.

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glycosylated extracellular loop. Using a variety of techniques, including surface plasmon resonance, co-immunoprecipitation and subcellular tracking, Demers et al. show that PCSK9 directly binds the extracellular loop of CD36 to mediate its internalization and degradation. Using PCSK9 gain and loss-of-function studies, the authors show that by altering CD36 cell surface expression and ligand uptake, PCSK9 has important functional consequences on fatty acid uptake by adipocytes and hepatic cells, and alters triglyceride accumulation in the liver.

PCSK9 interactions with CD36 have some notable differences from the well-described PCSK9-LDLR degradation pathway. First, although PCSK9 binds CD36 and LDLR with similar affinity at neutral pH (Kd ~1 μM), the PCSK9 residues involved in these interactions appear to be distinct. While PCSK9 binds to the epidermal growth factor-like repeat A domain of the LDLR through key amino acids found on the surface of its catalytic domain[4], previously identified gain (D374Y) and loss-of-function (F379A) PCSK9 mutations that alter this interaction do not affect PCSK9-CD36 binding affinity or CD36 degradation. Second, the fate of CD36 appears to differ from that of LDLR following PCSK9-mediated internalization. Upon internalization of the PCSK9-LDLR complex into endosomes, the binding affinity of PCSK9 for the LDLR increases several fold, thereby impeding LDLR recycling to the cell surface and diverting it to the lysosome for degradation. By contrast, PCSK9-mediated CD36 degradation in hepatic and adipocyte cell lines appears to involve both lysosomal and proteasomal pathways. While, the molecular mechanisms underlying CD36 targeting to the proteasome remain unclear, this divergence from the LDL-R degradation pathway suggests that different PCSK9 cargo can undergo distinct intracellular sorting upon internalization.

Despite the above noted differences in PCSK9-driven degradation of CD36 and LDLR, Demers et al report that a PCSK9 neutralizing antibody that inhibits its interaction with the LDLR also inhibits CD36 degradation, suggesting that PCSK9-targeted therapies may have additional effects on CD36 functions. Interestingly, studies in PCSK9 deficient mice showed that CD36 expression was only altered in select tissues. Whereas *Pcsk9*$^{-/-}$ mice showed no increase in CD36 expression in the intestine or heart, CD36 protein levels (but not mRNA) were strongly increased in the liver and adipose tissue as compared to control C57BL/6 mice. Notably, CD36 staining was specifically increased on the basolateral surface of hepatocytes with the loss of PCSK9. Conversely, injection of recombinant human PCSK9 to C57BL/6 mice rapidly reduced hepatic and adipose CD36 levels, whereas PCSK9 status did not expression of the close CD36 homologue SR-B1. Analysis of the metabolic effects of PCSK9 deletion on CD36-related functions revealed that *Pcsk9*$^{-/-}$ mice had greater hepatic uptake of a free fatty acid tracer, lipid droplet accumulation and triglyceride content (~4-fold increase). It should be noted that CD36$^{-/-}$ mice have increased liver fat: presumably this is due to a greater proportion of circulating fatty acids accumulating in the liver rather than peripheral tissues (adipose and muscles)[12]. These observations suggest that the liver does not require CD36 for fatty acid uptake in chow-fed mice. Thus, the role of CD36 in hepatocyte lipid metabolism is unclear and whether the increase in liver triglyceride observed by Demers et al. is directly a result of changes in CD36 rather than increased lipoprotein uptake cannot be determined.

What are the potential implications of these observations, especially as inhibitors of PCSK9 are being widely introduced into the clinic to treat patients with high circulating levels of LDL cholesterol? The work by Demers et al. adds CD36 to the list of receptors whose expression is controlled by PCSK9 and suggests that its inhibition might affect lipid metabolism in unforeseen ways. As in the case of the LDL receptor, PCSK9 degradation of CD36 is not universal, but appears to be limited to the adipose and liver. Why this occurs is unclear, but the tissue selective regulation by PCSK9 suggests that CD36 cell surface presentation or accessibility may vary. The data presented in mice suggest that deletion of PCSK9 allows greater adipose expression of CD36 and greater liver fat accumulation, but whether these effects are species specific remains to be determined. If this were also the case in humans, then use of recently approved PCSK9 antibody therapies to reduce LDL cholesterol may also increase adipose tissue and the development of non-alcoholic fatty liver. However, to date human studies using different PCSK9 antibodies have not shown a signal that liver function or glucose metabolism is altered with PCSK9 deficiency[5, 13]. A number of larger outcome trials are currently underway and further human data will become available. However, studies such as this will enlighten us on other important functions of PCSK9 and may direct our scrutiny of the developing clinical data.

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