PCSK9 and Lipoprotein(a)  

The Plot Thickens  

Peter P. Toth

Proprotein convertase subtilisin kexin type 9 (PCSK9) has rapidly become a focus of intensive investigation worldwide because of its linkage to multiple clinically important facets of hepatic lipoprotein metabolism. To facilitate secretion and proper folding, the zymogen pro-PCSK9 must undergo autocatalytic cleavage. Once in the extracellular space, mature PCSK9 does not engage in proteolytic activity because its active site is blocked by a prosegment.3 PCSK9 modulates the expression of a variety of cell surface receptors regulating lipoprotein and lipid trafficking across the cell membrane. Initial studies that lead to the identification of PCSK92 correlated 2 novel gain-of-function mutations in this enzyme with the phenotype of autosomal dominant hypercholesterolemia (familial hypercholesterolemia).1 Subsequent to these discoveries, loss-of-function mutations in PCSK9 were found to correlate with low serum levels of low-density lipoprotein cholesterol (LDL-C) and reduced risk for future cardiovascular events.4 Given these findings, PCSK9 has become an important therapeutic target in the management of dyslipidemia, and 2 monoclonal antibodies directed against it have recently been approved for use in patients with elevated LDL-C.

PCSK9 regulates the expression of a variety of other cell surface receptors. PCSK9 regulates the expression of the LDLR-related protein-1,7 the very-low-density lipoprotein receptor, and the apolipoprotein E receptor 2.8 In addition, PCSK9 has been shown to downregulate the expression of cluster of differentiation 36 (CD36), a transmembrane protein that functions as a fatty acid translocase in hepatocytes and adipocytes.9 PCSK9 increases lysosomal degradation of CD36 via a proteasome-dependent pathway. It is not yet established whether PCSK9 impacts LDL scavenging via CD36 by macrophage-derived foam cells. However, the decreased expression of these various non-LDLRs could potentially impact systemic clearance of remnant lipoproteins and fatty acids. Of great interest is the recent observation that a physical interaction between intracellular PCSK9 and apoprotein B (apoB) decreases the catabolism of apoB via an autophagosome/lysosome pathway and results in increased secretion of apoB lipoproteins.10 Overproduction or impaired catabolism of PCSK9 would thus be expected to exacerbate dyslipidemia by potentiating hepatic production and secretion of apoB-containing lipoproteins.

Are there any naturally occurring inhibitors or modulators of PCSK9 activity? It seems there are, although clearly the picture remains incomplete. Annexin A2 induces a conformational change in PCSK9, thereby reducing the functional activity of this enzyme and inducing increased expression of LDLR.11 In addition to binding to LDLR, PCSK9 can bind to LDL particles in plasma.12 It is estimated that 1 in 500 to 1000 LDL particles in plasma forms a complex with PCSK9.13 This sequestering of PCSK9 by LDL complex formation would result in indirect inhibition because less PCSK9 would be available along the hepatocyte cell surface to bind LDLR. Kosenko et al14 demonstrated that ≈40% of the total PCSK9 in circulation is bound to LDL particles. The binding of PCSK9 occurs via its N terminus, and kinetic studies are consistent with a single binding site on LDL. Hence, it is possible that LDL particles regulate their uptake by limiting serum levels of PCSK9. It remains to be determined if a specific subpopulation of LDLs with a proteome variant binds PCSK9 or if it is a random event with a fixed binding site. It is also of interest to establish whether some patients bare particular efficiency for PCSK9 sequestration by LDL particles. Furin is a serine protease that cleaves PCSK9 at Arg218, thereby converting its 62-kDa active form into a 55-kDa inactive one.15 The role of furin in PCSK9 regulation in vivo is not yet defined.

For >5 decades, it has not been clear how lipoprotein(a) (Lp(a)) is removed from the circulation. Rader et al16 demonstrated that Lp(a) clearance rates were identical between patients with homozygous familial hypercholesterolemia, heterozygous familial hypercholesterolemia, and normal controls, suggesting that Lp(a) uptake occurs via a pathway that is independent of LDLR. Lp(a) is highly atherogenic, and
its serum levels are genetically determined. Similar to other LDLs, it is found in atherosclerotic plaque. It may confer increased risk for thrombosis secondary to apolipoprotein(a) which has significant sequence homology to plasminogen. It has also been identified as an important carrier vehicle and delivery platform for oxidized phospholipids, which can boost intravascular inflammation and potentiate atherogenesis. Multiple prospective observational cohorts confirm that Lp(a) correlates highly with risk for myocardial infarction, ischemic stroke, aortic valve calcification, peripheral arterial disease, and mortality.\textsuperscript{17–19} Statin therapy has not been shown to attenuate the excess risk for cardiovascular morbidity or mortality attributable to elevations in Lp(a).\textsuperscript{20} Lipoprotein apheresis is an effective method for clearing Lp(a). Multiple types of molecular interventions for lowering Lp(a) are in development.

It is known that monoclonal antibodies directed against PCSK9 reduce serum levels of Lp(a) significantly.\textsuperscript{21,22} Recent investigation suggests that the PCSK9 monoclonal antibodies decrease Lp(a) via LDLR according to the following: (1) there are greater numbers of LDLRs to bind and clear Lp(a) particles and (2) by inducing dramatic reductions in LDL particles, there are more binding sites available with less kinetic competition for Lp(a) to bind to these sites.\textsuperscript{23} Romagnuolo et al\textsuperscript{24} demonstrated that overexpression of LDLR in HepG2 cells facilitates Lp(a) uptake via the LDL portion of Lp(a) and not its apolipoprotein(a) moiety. Moreover, similar to that for LDL, Lp(a) uptake was dependent on

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\caption{Proprotein convertase subtilisin kexin type 9 (PCSK9) and lipoprotein trafficking. Subsequent to release from the Golgi apparatus, low-density lipoprotein receptors (LDLR) translocate to the cell membrane where they are concentrated in clathrin-coated pits. Hepatocytes secrete PCSK9 into the extracellular space which can bind to the epidermal growth factor–like repeat A (EGF-A) domain of LDLR. Complexes composed of LDLR and LDL particles are internalized via endosomes. If the LDLR–LDL-P complex is bound with PCSK9, the complex is chaperoned into the lysosome for hydrolytic destruction. The LDLR in this case is not recycled to the cell surface. When LDLR–LDL-P complexes are not bound to PCSK9, the complex dissociates in response to a drop in pH within the endosome. The LDL-P is translocated to the lysosome for destruction, whereas LDLR is spared and recycled to the cell surface to initiate another round of LDL-P binding and uptake. The monoclonal antibodies directed against PCSK9 decrease LDLR translocation into the lysosome, increase LDLR surface expression, and significantly reduce plasma levels of LDL-P. Recent investigations demonstrate that PCSK9 can also regulate the expression of other cell surface receptors, such as a very-low-density lipoprotein receptor (VLDLR), the LDLR-related protein (LRP), an apoprotein E receptor, and cluster of differentiation 36 (CD36). In addition to its impact on cell surface receptor expression, PCSK9 potentiates the production of apo B and increases VLDL secretion by inhibiting the catabolism of apoB via an autophagosome/lysosome-dependent pathway. The serine protease furin (which is found in both membrane-bound and free forms) can cleave active, intact PCSK9 (62 kDa) into an inactive 55-kDa fragment. A small percentage of total circulating LDL and lipoprotein(a) (Lp(a)) can bind PCSK9. Although some Lp(a) is cleared by a pathway that is independent of LDLR, some is demonstrably cleared by LDLR.}
\end{figure}
clathrin-coated pits and a lysosome-dependent pathway. It is not yet known whether PCSK9 antagonism specifically reduces the production and secretion of Lp(a) or whether it facilitates Lp(a) clearance via lipoprotein cell surface receptors other than LDLR.

Is there more to the story? There has to be. In this issue of Circulation Research, Tavori et al12 hypothesized that because Lp(a) is a variant of LDL, it too binds and sequesters some amount of PCSK9 in serum. Interestingly, it does. In a cohort comprised dyslipidemic patients with Lp(a) mass concentrations of 39 to 320 mg/dL, these investigators convincingly demonstrated using ultracentrifugation, immunoprecipitation, and an ELISA that Lp(a) binds PCSK9 in serum. PCSK9 binds to apoB, and the binding is independent of the number of kringle IV-2 repeats or whether or not the Lp(a) contains the apolipoprotein(a) region that binds oxidized phospholipid, and there is a direct relationship between Lp(a)-associated PCSK9 and plasma Lp(a) levels. The PCSK9 associated with Lp(a) is intact and not hydrolyzed by furin. Fully one quarter of PCSK9 in plasma is associated with Lp(a) in these patients with elevations in this lipoprotein. At least in patients with elevated Lp(a), PCSK9 seems to have some preferential binding (1.7-fold higher) for Lp(a) compared with LDL. However, with only 1 out of 175 Lp(a) particles binding PCSK9, this represents a very small percentage of total Lp(a) that is complexed. In patients with lower plasma levels of Lp(a), this percentage would be expected to be still lower. Does this represent passive, random association? Is it a means of sequestering PCSK9 and rendering it incapable of binding to LDLR, or does it constitute a tagging of sorts to mark a small subpopulation of Lp(a) particles for a different metabolic fate? We do not know yet.

The functional significance of the association between PCSK9 and Lp(a) cannot be gleaned from these data. The PCSK9 monoclonal antibodies are approved for clinical use in the United States and Europe. To date, they have demonstrated an acceptable level of safety and reduce atherogenic lipoprotein burden in serum to a remarkable degree. The results of cardiovascular outcome trials with these agents are eagerly awaited. Clearly, our knowledge, although advancing rapidly, is rudimentary, and there is much about the molecular dynamics of PCSK9 that we still do not know. Given the growing, important role these agents are expected to play in rapidly, is rudimentary, and there is much about the molecular dynamics of PCSK9 that we still do not know. Given the growing, important role these agents are expected to play in the management of patients with dyslipidemia, it is imperative that further investigation continues to more fully sort out the comprehensive range of PCSK9’s activity in all tissues (especially the liver and intestine) in which it is expressed, how its antagonism impacts lipoprotein trafficking in plasma and lipid/lipoprotein metabolism in specific cell types, and how different tissues may engage in molecular cross talk secondary to increases or decreases in PCSK9 functionality. You can expect the results to be both fascinating and marvelously surprising.

Disclosures
Dr Toth is a member of the speaker’s bureau for Amgen, Amarin, Kowa, Merck, Regeneron-Sanofi and consultant to Amgen, Amarin, AstraZeneca, Kowa, Merck, and Regeneron-Sanofi.

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