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Original Article

Posttranslational modification of proprotein convertase subtilisin/kexin type 9 is differentially regulated in response to distinct cardiometabolic treatments as revealed by targeted proteomics

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BACKGROUND: The proprotein convertase subtilisin/kexin type 9 (PCSK9) is a secreted protein that interacts with the low-density lipoprotein (LDL) receptor at the surface of hepatocytes to regulate circulating LDL cholesterol levels. High circulating PCSK9 levels have been associated with elevated LDL cholesterol. Recently, the Food and Drug Administration of the United States approved new LDL cholesterol–lowering drugs that specifically target the inhibition of PCSK9. Similar to most human proteins, PCSK9 exists in multiple forms as it is the target of posttranslational modifications (PTMs) such as proteolytic cleavage, phosphorylation, and others, which can affect its biological activity. However, commercially available assays, such as enzyme-linked immunosorbent assays, do not discriminate between these forms.

OBJECTIVE: To investigate, in 2 patient cohorts, the relationships between circulating levels of multiple forms of PCSK9 and cardiometabolic interventions or treatments known to reduce LDL cholesterol levels.

METHODS: PCSK9 forms were measured in plasma: (1) in 20 patients before and 6 months after bariatric surgery and (2) in 132 patients before and 12 months after daily statin treatment. A series of specific peptides used as surrogates for various PCSK9 forms were quantified by a novel semiautomated proteomic assay termed protein affinity capture coupled to quantitative mass spectrometry.

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RESULTS: Bariatric surgery resulted in a decrease in the plasma level of PCSK9 prodomain ($P < .05$), but did not result in a significant change in other measured PCSK9 forms. Statin treatment resulted in an increase in all measured plasma PCSK9 peptides ($P < .001$), but a 25% decrease in the phosphorylated state of PCSK9 at S688 ($P < .05$).

CONCLUSIONS: These unexpected findings indicate that measuring the circulating levels of the various domains and PTMs of PCSK9 provides more in depth information than total PCSK9 and that the prodomain and the phosphorylated state of S688 may represent novel biomarkers to explore in cardiometabolic diseases and response to treatment. In addition, our data generated new hypotheses on the function of PCSK9 PTMs in health and disease.

Introduction

The proprotein convertase subtilisin/kexin type 9 (PCSK9)[1] is a secreted protein that binds the low-density lipoprotein (LDL) receptor (LDLR) thereby promoting its degradation in endosomes/lysosomes and the accumulation of LDL cholesterol (LDL-C) in the blood circulation. It is the target of 2 recently Food and Drug Administration of the United States-approved monoclonal antibodies to treat some patients with familial hypercholesterolemia or clinical atherosclerotic cardiovascular disease when diet and maximally tolerated standard cholesterol-lowering therapy only lead to suboptimal LDL-lowering levels. Very recently, the long-awaited results of the Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) outcome trial showed that PCSK9 inhibition not only decreased LDL-C by ~60% but also significantly reduced by 15% to 20% the risk of cardiovascular events in patients with atherosclerotic cardiovascular disease.[2]

Like most human proteins with posttranslational modifications (PTMs), PCSK9 exists in multiple forms with varying biological activities (Fig. 1A). The zymogen proPCSK9 is autocatalytically cleaved between Q152 and S153 in the endoplasmic reticulum to produce a prodomain (aa 31–152) and a mature protein (aa 153–692) that stay noncovalently associated and are secreted as an enzymatically inactive complex.[1,3] This autocleavage is a prerequisite for PCSK9's exit from the endoplasmic reticulum and secretion, as well as for its activity in enhancing LDLR degradation.[1,10] Of note, the prodomain appears to play a partial inhibitory role on the PCSK9-stimulated degradation of LDLR as mutants lacking the N-terminal acidic sequence of the prosegment (aa 31–58) show a 4- to 7-fold higher activity on LDLR degradation.[11,12] PCSK9 is also cleaved by the enzyme furin at R218, which leads to an N-terminal segment (aa 153–218) and a truncated PCSK9 (aa 219–692) that is believed to be inactive,[5–8,13] or to show reduced activity[9] toward LDLR degradation. The prodomain appears to dissociate from PCSK9 after furin cleavage.[7,8] However, whether the prodomain and N-terminal segment of furin-cleaved PCSK9 are degraded after dissociation with PCSK9 is not clear. Other PTMs have been identified on PCSK9 such as sulfation at Y38,[8] phosphorylation at S47 and S688,[14] and N-glycosylation at N533.[1,10] It was first suggested that an unidentified Golgi casein kinase–like kinase was responsible for PCSK9 phosphorylation at S47 and S688.[14] FAM20C was later identified as the bona fide Golgi casein kinase that phosphorylates PCSK9 at these 2 positions.[15] We have recently reported that PCSK9 circulates in human plasma with S688 phosphorylated at high stoichiometry.[16] Phosphorylation of S47 has been suggested to protect the prodomain from proteolysis in HuH7 cells[14] and, although not peer reviewed, a patent application published by a team from the Ottawa Hospital Research Institute[17] reports that S688 or S47 phosphorylation could both stimulate PCSK9's activity toward LDLR degradation in HuH7. Nonetheless, published data on the physiological roles of PCSK9 phosphorylation remains scarce.

One reason for this lack of information is the fact that until recently, the available assays used to quantify circulating PCSK9 in humans (eg, enzyme-linked immunosorbent assay [ELISA]) only provided total protein values without discriminating between its various domains, forms, and PTMs. To address this, we recently reported a protein affinity capture coupled to quantitative mass spectrometry assay (PAC-qMS, also known as mass spectrometric immunoassay coupled to selected reaction monitoring) that allows the simultaneous semiautomated high-throughput measurements of a series of peptides as surrogates of each circulating plasma PCSK9 domains as well as the nonphosphorylated and phosphorylated peptides encompassing S688.[16] Similar approaches have been used by other laboratories to quantify the proteoforms of serum amyloid A,[18] osteocalcin,[19] apolipoprotein C-I, C-II, and C-III[20] after immune-enrichment and top-down matrix-assisted laser desorption/ionization-time of flight, a different mass spectrometric method. We have now used our PCSK9 PAC-qMS assay in patient cohorts to assess the relationships between various PCSK9 forms and 2 different cardiometabolic treatments that are known to reduce cholesterol levels, namely statin therapy and bariatric surgery. We hypothesized that these LDL-lowering treatments would lead to differential effects on specific PCSK9 forms as opposed to total PCSK9.

Methods

Chemicals and reagents

Recombinant human PCSK9 (R&D Systems) was resuspended according to the manufacturer's recommendations, aliquoted for single use and stored at −80°C until analysis.