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Review

Biological and analytical considerations for PCSK9; A key target for LDL-cholesterol lowering

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ABSTRACT

Unraveling the role of proprotein convertase subtilisin kexin type 9 (PCSK9) as a key post-transcriptional regulator of the LDL receptor (LDLR) has shed light on its attractiveness as a target for LDL cholesterol (LDL-C) lowering. PCSK9 is a serine protease that is secreted by the liver upon post translational self-cleavage of its pro-domain. Once in circulation, the protein binds the hepatic LDLR, and upon internalization, the receptor is sequestered for lysosomal destruction. Gain-of-function mutations for PCSK9 induce familial hypercholesterolemia, whereas loss-of-function mutations display a broad range of mutation dependent LDL-C lowering. Initial mutation and knockout analyses established the correlation between PCSK9 and circulating LDL-C. Ensuing RT-PCR studies demonstrated statin induced up-regulation of PCSK9 mRNA expression. Subsequently, the development of novel monoclonal PCSK9 antibodies facilitated sensitive and robust ligand-binding assays that have produced concrete conclusions linking PCSK9 to LDL-C regulation. These methods have demonstrated the impacts of current lipid lowering therapies on levels of circulating human PCSK9; furthermore, they will be a key component in addressing the pharmacodynamic responses of first generation PCSK9 lowering therapeutics. Here, we summarize and assess the methodologies for PCSK9 measurement in the context of a review of events that have led to our present knowledge about this intriguing target. Moreover, we address conflicting data and discuss ongoing bioanalytical challenges in understanding the true circulating levels of PCSK9. Finally, we discuss the pharmacological impact of PCSK9 measurement as a screening tool and a biomarker for future LDL-C lowering therapeutics.

KEYWORDS: PCSK9, statins, LDL-cholesterol, LDL receptor, fibrates

INTRODUCTION

In less than a decade, proprotein convertase subtilisin kexin type 9 (PCSK9) has become a well-characterized novel target for lowering LDL-cholesterol (LDL-C) [1-5]. Discovered in 2003 as the ninth member of the proprotein convertase family, PCSK9 is synthesized in liver, intestinal and brain tissues as a 72 kD zymogen that is activated during secretion upon calcium independent autocatalytic cleavage of its 14 kD pro-domain [6-8]. Once in circulation, mature PCSK9 remains bound to its pro-domain at the active site, and is not known to have enzymatic activity beyond its self-cleavage [9-10]. While it was initially thought that PCSK9 might be associated with lipoproteins in circulation, experimental evidence suggests that it is likely free of such associations [11]. Like the LDL-receptor (LDLR), transcription of PCSK9 is mediated by the transcription factor sterol regulatory element binding protein 2 (SREBP-2) and is thus regulated by intracellular hepatic cholesterol levels [12-13]. Soon after its discovery, PCSK9 became a major gene of interest when it was observed that

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certain mutations had a profound impact on circulating LDL-C levels.

Familial hypercholesterolemia (FH) is an inherited disorder of lipid metabolism characterized by LDL-C levels two times that of the normal population with bimodal distribution [14-15]. In 1974, it was shown that FH is the result of deficits in removal of LDL-C from plasma [16]. Since then, three loci have been connected with the disorder. The first was a defect in the gene for LDLR. The next was R3500Q in the apoB100 gene. And the most recent was found to be located on 1p34.1-p32 via a French family (HC2) with no linkage to either LDLR or apoB100 anomalies [14]. Given the chromosomal location and the anatomical localization of PCSK9 mRNA expression, Abifadel and colleagues studied the third locus, which contained 41 genes, including PCSK9. Using parametric linkage analysis, they found two missense mutations [17]. The first, S127R, was near the PCSK9 zymogen processing site, while the second, F216L, was determined to be close to the active site of PCSK9 [17]. Later, another gain of function mutation was discovered - D374Y - which increases the affinity for LDLR tenfold [15, 18-19].

The location of PCSK9 expression was similar to other genes that are involved in cholesterol biosynthesis. As a result, microarray studies were performed which showed that PCSK9 mRNA was downregulated in mice fed with increased dietary cholesterol [13]. In 2004, Dubuc and colleagues found that statins upregulate PCSK9 mRNA [20] by using quantitative RT-PCR, to measure PCSK9 mRNA levels in both human hepatic (HepG2) cells and in human primary hepatocytes. These findings would later be supported by studies that looked at the effect of statins on PCSK9 expression levels.

Gain-of-function mutations illustrated a distinct relationship between PCSK9 and cholesterol metabolism. To begin elucidating the mechanism behind this connection, Maxwell and colleagues demonstrated that adenoviral over-expression of PCSK9 in mice induced a five-fold increase in non-high density lipoprotein cholesterol (HDLC). Interestingly, LDL-receptor (LDLR) knockout mice did not exhibit an increase in cholesterol as a result of PCSK9 overexpression. Furthermore, overexpression of PCSK9 reduced the mouse LDLR protein concentration to negligible levels, while having no impact on LDLR mRNA [21]. These data were the first to demonstrate that PCSK9 inhibits LDLR function at the protein level. Researchers have since determined that upon self-cleavage and secretion, PCSK9 binds the LDLR, forming a complex which is then internalized into the hepatocyte by LDLR mediated endocytosis. By an unclear mechanism, the complexed LDLR's are restricted from being recycled to the cell surface, and are targeted for destruction within acidic intracellular lysosomal compartments [10, 18, 22-31]. These data suggested that increased PCSK9 results in degradation of LDLR protein, which ultimately leads to the observed impairment of LDL-C clearance. Figure 1 illustrates the fate of the LDLR under circumstances with and without PCSK9 binding.

Increasing interest in PCSK9 as a target for LDL-C lowering

For many of these early efforts, PCSK9 expression was measured at the mRNA level by quantitative RT-PCR, and correlated with lipid profile parameters and measurements of LDLR protein (western blotting) and LDLR mRNA (RT-PCR). The first western blotting assays to measure PCSK9 protein concentration were intended to provide confirmation and quantfication of expression in engineered models [21, 32-34]. Modified vectors induced overexpression of PCSK9, and it was expected that levels within such a system would sufficiently increase and be easily detectible. These methods utilized either polyclonal antibodies to immunogenic peptide fragments of PCSK9 or they measured V-5 or FLAG tags that were inserted into the PCSK9 cDNA for adenoviral vector subcloning. The methods were semi-quantitative, usually employing a surrogate protein such as tubulin to establish normalized outputs. In these instances, western blotting methods provided a reliable means of assessing percent increases in PCSK9. Addressing clinical aspects of PCSK9 however, would require the ability to measure the endogenous circulating protein.

After intriguing researchers as a newly discovered gene with implications around FH, interest in PCSK9 grew exponentially in 2006 when the first

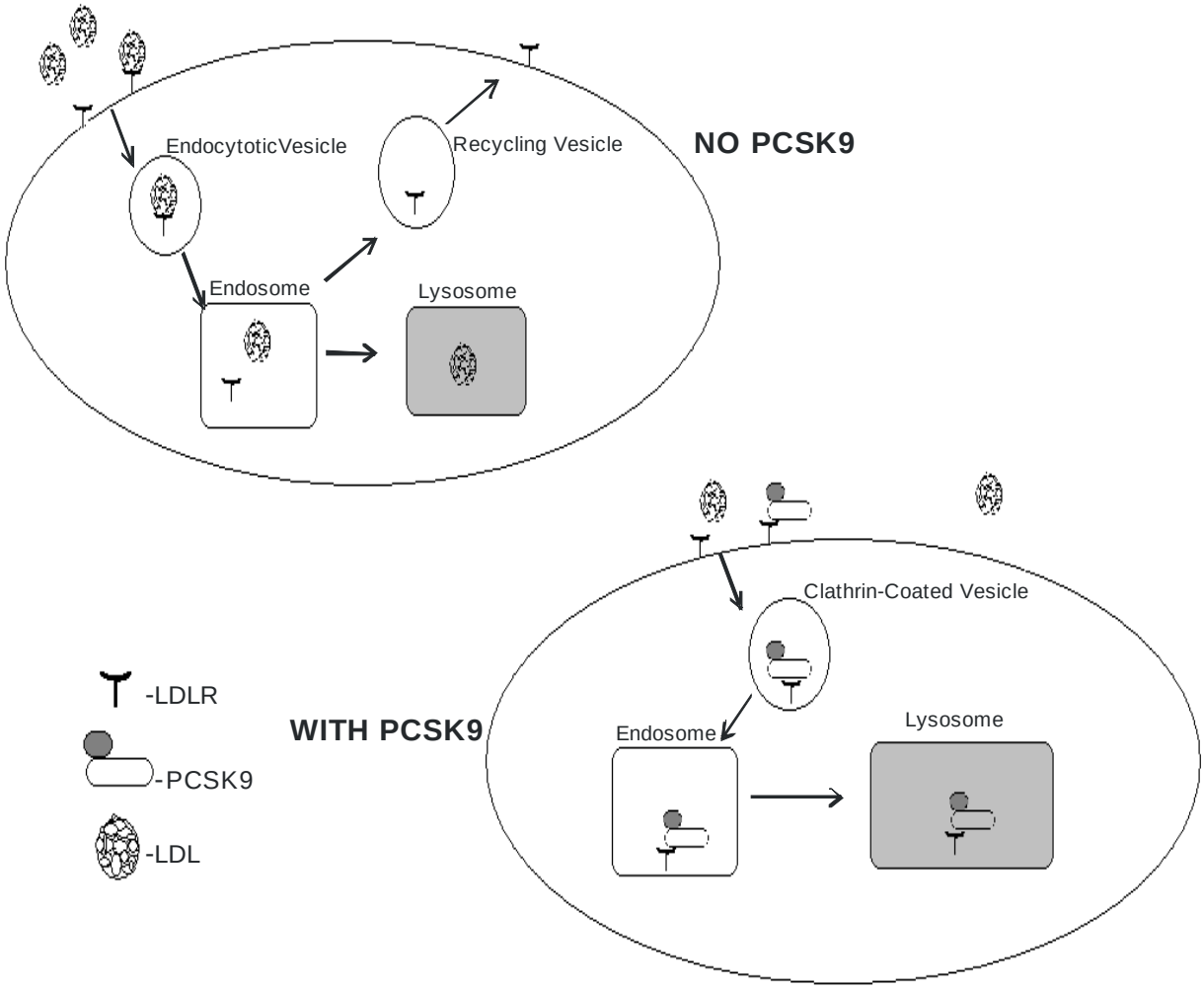

Figure 1. Potential outcomes for hepatic LDLR. In the upper left (labeled "No PCSK9"), circulating LDL-C binds to LDLR. This complex is then internalized and sent to the endosome where the proteins disassociate. The LDLR is recycled to the surface of the hepatocyte for further LDL-C binding. In the lower right (labeled "With PCSK9"), PCSK9 binds to the EGF-A domain of LDLR. This complex is internalized via clathrin-coated vesicles and taken to the endosome. Unlike the LDL-LDLR moiety, PCSK9-complexed LDLR are sequestered for lysosomal degradation, resulting in decreased cell surface LDLR and increased LDL-C.

loss-of-function (LOF) mutations were reported. Cohen and colleagues discovered two nonsense mutations (Y142X and C679X) in the African American population that inhibited secretion of the protein [35]. These mutations caused a profound lowering of LDL-C, and this lowering effect on LDL-C was statistically correlated in a large prospective study to an 88% decrease in incidence of coronary heart disease (CHD). Following this observation, several additional loss-of-function mutations were described [32, 36-38]. Individuals possessing loss-of-function mutations presented with significantly lower LDL-C, and some studies demonstrated increased statin response. Extreme cases, including a compound heterozygote for such LOF mutations were identified and were found to have negligible levels of circulating PCSK9 and extremely low LDL-C [39, 40]. These population based analyses represented a milestone, as circulating plasma PCSK9 was measured by immunoprecipitation (IP) and western blotting. Zhao and colleagues utilized a (protein G-coupled) rabbit polyclonal anti-human PCSK9 antibody to precipitate PCSK9 from human plasma and a

newly described c-terminal specific monoclonal anti-human PCSK9 antibody as a detection reagent for western blotting [39]. This approach enabled identification of the aforementioned compound heterozygote by detecting a distinct PCSK9 drop in one generation of a familial pedigree analysis. Furthermore, they were able to effectively demonstrate that PCSK9 can be measured in human plasma. Figure 2 provides a detailed diagram signifying the locations of several known mutations within PCSK9.

Quantitative measurement of circulating PCSK9

The loss of function observations validated PCSK9 as a major therapeutic target for inhibition. Consequently, gaining an accurate assesment of PCSK9 physiology at the protein level became imperative. In November of 2006, Lagace and colleagues developed a sandwich ELISA capable of quantifying human plasma PCSK9 [18]. The capture antibody was a murine monoclonal antibody to the catalytic domain of the protein, while the detection antibody was a rabbit polyclonal antibody directed at two immunogenic peptides also found within the catalytic domain. Standardization and quantification were performed using purified PCSK9. Analysis of human plasma from 72 volunteers demonstrated that circulating PCSK9 levels were in the range of 50-600 ng/mL with the highest frequency falling in the ~100-350 ng/mL range. Soon thereafter, our own laboratory developed a dual monoclonal sandwich ELISA that utilized capture and detection antibodies directed against independent epitopes at the c-terminal domain of PCSK9 [11]. Standardization was achieved using recombinant PCSK9 (rPCSK9) expressed in stable HEK293 cells, and serum was defined as the matrix of choice. We utilized our assay to assess the correlation between

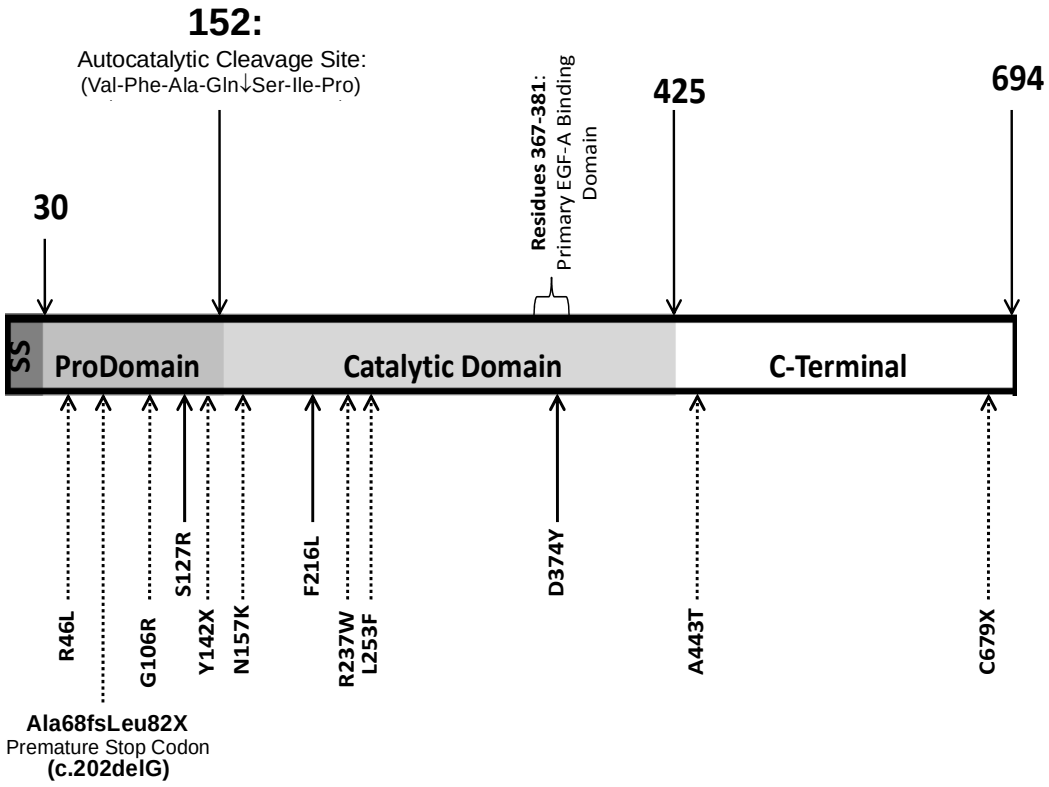

Figure 2. Diagram of PCSK9 detailing proteomic domains and locations of mutations. Mutations denoted with dashed arrow signify loss of function mutations. Mutations denoted with solid arrows signify gain of function mutations. Residues 367 - 381 are known to be the primary binding domain of the EGF-A interaction with LDLR. SS signifies the signal sequence of the protein.

circulating PCSK9 and LDL-C in a donor population of 55 healthy subjects with a broad spanning lipid profile. Resulting levels of PCSK9 ranged from 11 ng/mL to 115 ng/mL and were directly correlated with serum LDL-C (r=0.45, P=0.0003). While this correlation appeared to be lower than originally expected, this study had not taken into account whether participants were taking lipid-lowering drugs. Based upon earlier reports of statins significantly increasing PCSK9 mRNA, it was hypothesized that introduction of statin use would considerably alter any correlation between PCSK9 and LDL-C.

Impacts of current lipid lowering therapies

As the current benchmark for LDL-C lowering, statins are the most prescribed class of lipid lowering therapies. Statins inhibit 3-Hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase) thereby disrupting the cholesterol biosynthesis pathway [41]. This decrease in cholesterol synthesis initiates increased SREBP2 activity, thus up-regulating both LDLR and PCSK9 expression. Resultant up-regulation of LDLR levels facilitates greater LDL-C clearance, thus providing the mechanism for cholesterol lowering [42]. It was postulated that increased PCSK9 may counteract this effect to some extent by neutralizing the activity of the LDLR. Using the aforementioned ELISA method, our laboratory was able to demonstrate that the circulating levels of PCSK9 increased significantly in patients that were prescribed statins. At a 40 mg/day dose of atorvastatin, serum PCSK9 was increased 34%, while LDL-C was lowered 42%, thus abolishing the correlation between them [42]. Several other laboratories confirmed these findings, and the collective data suggested a dose dependent effect [43-45]. We later demonstrated that high dose atorvastatin (80 mg/day) induced rapid increases in PCSK9 and that these elevated levels were sustained over the course of a 16 week dosing period [46]. This sustained dose-dependent increase in PCSK9 began to explain the attenuation of statin efficacy that accompanies increased dosing.

While researchers drew similar conclusions around the statin effect on PCSK9, this has not been the case in addressing the impacts of fibrates. Fibrates are a class of peroxisome proliferator-activated receptor alpha (PPARα) agonists. Known for their ability to lower plasma triglyceride levels, fibrates are often also utilized for their HDLC raising and LDL-C lowering properties [47]. Much like the focus on statin influence, researchers became interested in understanding how fibrates affect PCSK9 levels. Individual preferences in analytical techniques and selected models however, have created some discrepancies around the effect of fibrates on PCSK9. In early 2008, Kourimate, and colleagues utilized western blotting with a rabbit polyclonal antibody to examine PCSK9 response to fibrates in immortalized human hepatocytes. Their findings indicated decreases in both cellular and secreted PCSK9 levels with concomitant increases in PC5/6A and furin expression [48]. These data suggested that fibrates operate via an alternative mechanism to statins. In the following months however, conflicting reports surfaced around the fibrate effect in humans.

First, Lambert and colleagues disclosed the development and utilization of a sandwich ELISA using two commercially available polyclonal PCSK9 antibodies to analyze the effect of fenofibrate in a cohort of 115 diabetic patients. Their report suggested an 8.5% decrease in PCSK9 in response to fenofibrate treatment (200 mg/day) over a 6-week period. Interestingly, the described circulating levels in this study were upward of ten-fold greater than those previously proposed by other laboratories [49]. Soon thereafter, Mayne and colleagues reported their use of immunoprecipitation (IP) and western blotting to show that fibrates increased PCSK9 approximately 17% in 19 human subjects. The IP was performed using a rabbit polyclonal antibody, and the immunoblotting protocol utilized an anti-rPCSK9 provided by Seidah and colleagues [43]. Again, the reported levels (1-6 µg/mL) were higher than what have typically been reported, however, this could have been attributed to their method of detection and standardization. This laboratory also stated that they saw no fibrate effect when dosing was performed in HepG2 cells, suggesting that clinical applications around PCSK9 measurement are most applicable in human *in vivo* studies. As the effect of fibrates remained in question, our laboratory decided to examine serum PCSK9 levels from a previously described clinical trial cohort of hyperlipidemic patients. Subjects in this study had been dosed with fenofibrate (200 mg/day) for 12 weeks, and blood was drawn at baseline and

endpoint. Upon analyzing the samples with our described ELISA, we noted a 25% increase in PCSK9 as a result of the treatment [50]. Soon thereafter, Costet and colleagues confirmed our findings using a commercially available ELISA to highlight a 26% increase in PCSK9 levels in 26 diabetic patients following 6 weeks of fenofibrate (160 mg/day) treatment [44]. While earlier outcomes were mixed, these most recent data were based upon robust ELISA methods with established histories. Therefore, it appears that fibrates do initiate a significant increase in PCSK9 levels. It is possible that fibrate induced cholesterol-lowering initiates the SREBP2 pathway, thus indirectly increasing PCSK9 levels by a mechanism similar to that of statins.

Methodological challenges

While western blotting has proven to be a crucial analytical technique in the characterization of PCSK9, our laboratory has noted some caveats to using this as a means in which to quantify PCSK9 levels. Secreted PCSK9 migrates at ~64 kD on a non-reducing, non-denaturing gel, and therefore must be immunoprecipitated from serum or plasma to avoid co-migration with the high abundance (~4 g/dL) of circulating albumin. While immunoprecipitation will sufficiently alleviate this concern, there is a level of variability added in the necessary steps to dissociate and elute the antigen from the antibody coupled matrix (i.e; agarose or protein G beads) [11]. Furthermore, from a quantitative standpoint, standardizing to a recombinant or purified protein by methods of densitometry is typically considered a semi-quantitative practice. As such, we have frequently used IP/western blotting to visually confirm biological trends established by ELISA measurement [11, 42, 50]. This approach has also been particularly useful in screening antibodies for ELISA method development.

It was IP/western data that initially alerted us to a smaller isoform of PCSK9 that consistently appeared as a band of varying intensity at ~55 kD. Bennjannet and colleagues thoroughly characterized this "lower" band as an inactive variant of PCSK9 that is the result of membrane bound furin or intracellular PC5/6A cleavage at Arg^{218} [51-52]. This cleavage takes place within the PCSK9 secretory pathway, and gain-of-function mutations have been characterized as having a sequence that prohibits this cleavage from taking place [52]. In our own analysis, we assessed whether antibodies were capable of measuring this shortened variant by IP/western blotting. As Figure 3 illustrates, the lower band (inactive variant) was prominent when we used a polyclonal antibody directed at full-length PCSK9 to immunoprecipitate the protein. Analytically, these findings suggest that new approaches to measuring PCSK9 that would allow for the measurement of the ratio of the active to inactive isoforms might be possible. Following their characterization of this inactive variant, Dubuc and colleagues reported the development of an ELISA that measured total circulating PCSK9 [53]. These results suggest that ELISA methods that measure total and active variants of the protein should be possible by using different combinations of C-terminal and catalytic domain antibodies.

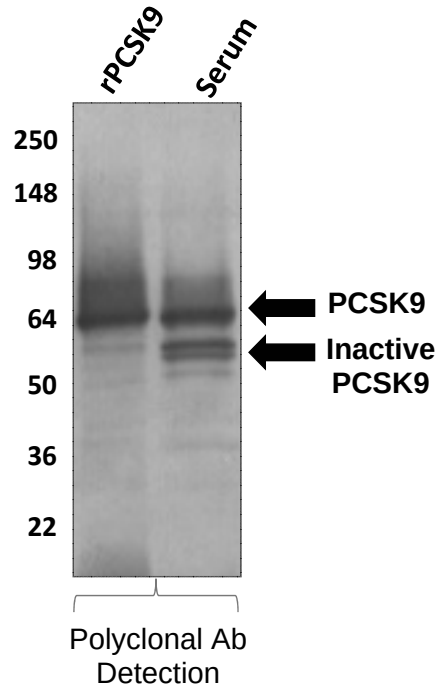

Figure 3. Immunoprecipitation and western blotting of circulating PCSK9. Human serum was immunoprecipitated using an anti-C-terminal PCSK9 antibody. Captured PCSK9 was eluted and separated electrophoretically, and the migrated protein was transferred to nitrocellulose for western blotting. Detection was performed with anti-full length polyclonal antibody.

To date, many research laboratories have reported development and/or use of methods that measure circulating human PCSK9 [11, 39, 43-44, 48-49, 54]. Table 1 provides a comparison of several methods that have been reported in the literature. While the range of reported levels is quite broad, the general consensus is that PCSK9 circulates at relatively high levels and is modulated by several mutations and known therapeutic entities. Typically, laboratories have reported levels in the low-to-mid hundreds of nanograms per milliliter of plasma. Although, Lambert and colleagues initially reported levels ranging from 100-9300 ng/mL, a more recent article from this group suggests that they saw levels aligned closer to 100 ng/mL (mean = 89.5) [49, 54]. Some of this variation could be attributed to changing their capture antibody, while it could also simply reflect the variability in their donor pools. Recent data from Lakoski and colleagues suggest that there is likely a dramatic range in normal circulating levels that can reach up to ~3000 ng/mL. To demonstrate this, they employed an assay that captured with a polyclonal antibody, and detected with an equimolar mixture of two monoclonal antibodies directed at the c-terminus and the catalytic domain, respectively. This method would appear to measure total PCSK9, as the polyclonal capture is directed at full length PCSK9 [55].

ELISA and IP/western blotting have served as the two techniques for measurement of circulating PCSK9, however, ELISA is generally accepted as setting a higher standard for both precision and accuracy when quantitative output is desired. That being said, not all ELISA's are created equal given the many variables that must be taken into consideration during their development. Regarding PCSK9, we have noticed several caveats. One is that some antibodies appear to pose a significant difference in their binding of recombinant PCSK9 versus the endogenous protein. This introduces obvious concerns around establishing absolute circulating levels, and it is an issue that can be difficult to overcome. The majority of rPCSK9 protein standards described in the literature are expressed from HEK293 stable lines. It is possible that there are endogenous post-translational modifications that are not easily replicated in these expression systems. A method for measuring PCSK9 by mass spectrometry with a viable internal standard might provide a means of better detecting and quantifying small differences in the protein. This approach could provide a path forward for establishing absolute values while also providing

Table 1. A detailed comparison of published methods for measuring human PCSK9.

Author	Method	Capture Ab	Detection Ab	Standard (quantitation)	[PCSK9] (ng/mL)
Lagace *et al.*	ELISA	MAb (Cat. Domain)	Rabbit polyclonal	rPCSK9 (HEK293)	50-600
Alborn *et al.*	ELISA	mMAb (c-terminus)	mMAb (c-terminus)	rPCSK9 (HEK293)	11-115
Lambert *et al.*	ELISA	Goat polyclonal	Rabbit polyclonal	rPCSK9 (HEK293)	100-9300
Costet *et al.* (Cyclex Co, Japan)	ELISA kit (Circulex)	Polyclonal	Monoclonal KS-2C10	rPCSK9	158-318
Dubuc *et al.*	ELISA	Rabbit polyclonal	Rabbit polyclonal	rPCSK9 (HEK 293)	35-225
Baass *et al.*	ELISA	Rabbit polyclonal	Rabbit polyclonal	rPCSK9 (HEK293)	89.5 (mean)
Lakoski *et al.*	ELISA	Rabbit polyclonal	MAb (Cat. Domain) MAb (c-terminus)	rPCSK9 (HEK293)	33-2988
Mayne *et al.*	western blot	Rabbit polyclonal Anti-native (IP)	Rabbit polyclonal Anti-rPCSK9	Quantified by densitometry	6100 (mean)

a means of comparison for higher throughput ELISA methods. We have also seen differences in antibody recognition between His-tagged and non-tagged recombinant standards. Additionally, we remain cognizant of the possibility that multimerization of the standard and/or the endogenous protein is possible. To address this concern, we have paired monoclonal antibodies with themselves in a sandwich format to measure endogenous and recombinant protein, and they gave signal that was comparable to background (not published). This suggests that multimerization is unlikely, although this possibility cannot be ruled out.

The future of PCSK9 as a therapeutic target

Given the knowledge that certain loss-of-function mutations can lead to LDL-C levels as low as 14 mg/dL, potential therapeutics which can neutralize PCSK9 functionality appear to be promising [39]. Initial approaches utilized DNA or mRNA interference methods via anti-sense single oligonucleotides (ASO) to interfere with transcription of the protein. Graham and colleagues described the use of 2nd generation antisense single oligonucleotide (ASO) interference in mice fed a high fat diet [56]. This approach resulted in a 92% reduction of PCSK9 mRNA within 6 weeks, while resulting in a greater than 2-fold increase in functional LDLR protein. Similarly, implementation of both single and once-weekly repeated dosing of locked nucleic acid antisense oligonucleotide demonstrated a 95% reduction in PCSK9 mRNA with a concomitant 2 - 3 fold increase in LDLR protein *in vivo* [57].

While the development of anti-PCSK9 antibodies has advanced the ability to quantify circulating levels of PCSK9 protein, similarly antibodies have been generated which have been shown to neutralize the effect of PCSK9 both *in vitro* and *in vivo*. These antibodies target one of two areas - either they prevent the binding of LDLR and PCSK9 or they prevent the internalization of PCSK9 [57]. The primary approach has been to develop antibodies which inhibit PCSK9 - LDLR binding. Zhang and colleagues elucidated that the epidermal growth factor like repeat A [EGF(A)] of low density lipoprotein receptor is involved in the binding of PCSK9 to LDLR [25]. As a result, this region of the protein has been targeted by many groups. For example, Ni and colleagues demonstrated that an antibody which mimics the EGF(A) domain provides a 70% decrease in free PCSK9 *in vivo*, however, the total levels of PCSK9 remained unchanged [58]. Chan and colleagues similarly developed an antibody which interfered with the PCSK9 -LDLR interaction. They went a step further however, and developed an antibody which not only targeted recombinant PCSK9, but also the gain-of-function mutation D374Y, thereby making it desirable for addressing at least one form of genetic hypercholesterolemia [59]. Using both mice and nonhuman primates this group also demonstrated that their antibody could work in conjunction with statins to lower LDL, and that there was a rapid, yet reversible decrease in total cholesterol following a single injection.

Lastly, there has also been a focus on small molecule inhibitors for PCSK9, two examples of which are berberine and farnesoid X receptor (FXR) agonists. Both molecules work to inhibit the translation of PCSK9 by interference within the promoter region. Berberine, which interferes through the SRE and HNF1 regions of the promoter, has been shown to decrease PCSK9 mRNA by 77% in HepG2 cells [60-61]. This decrease in mRNA was accompanied by an 87% drop in PCSK9 protein. Berberine has also been shown to suppress statin-induced increases in PCSK9 mRNA. Alternatively, FXR has been targeted by both chenodeoxycholic acid and the FXR agonist GW4064. Again working at the translational level, expression of PCSK9 is decreased by 59% in human hepatocytes [62]. More importantly, both of these agonists decrease both intracellular and extracellular PCSK9.

CONCLUSIONS

PCSK9 has become an attractive therapeutic target due to the novel, post-transcriptional mechanism by which it modulates LDLR protein levels and ultimately regulates LDL-C. Moreover, nature has produced individuals with PCSK9 loss-of-function mutations that present with extremely low LDL-C and no known adverse health risks. While the evolutionary purpose of PCSK9 is yet to be fully understood, researchers have demonstrated that

PCSK9 appears to have a physiological role in maintaining consistent LDL-C levels. Two laboratories have now shown that PCSK9 is tightly correlated with lathosterol, a known marker for hepatic cholesterol biosynthesis, in normal and fasting (1-7 day) states [63-64]. While the levels of PCSK9 and lathosterol demonstrated marked diurnal variation and significant fasting imposed decreases, LDL-C concentrations remained consistent.

The capability of measuring circulating PCSK9 has brought significant validation to its potential both as a therapeutic target and as a biomarker for LDL-C-lowering therapies. ELISA data indicate that common lipid lowering therapies increase PCSK9, thereby curtailing their potential maximal efficacy. These data suggest that negative modulation of PCSK9 would likely provide unique and additive benefits to at least two popular classes of LDL-C lowering medicines. Current ELISA methods have allowed us to solidify the correlation between PCSK9 and LDL-C lowering at the protein level. Furthermore, they have played key roles in unlocking new mutations, while allowing researchers to better understand the physiological roles of the protein.

These methods have already established PCSK9 as a novel, circulating biomarker for screening future LDL-C lowering compounds. As previously discussed, efforts are well underway to develop molecules that will neutralize, inhibit, or prevent the transcription of PCSK9. Clinical trials for such efforts will be able to call upon LDL-C as the endpoint biomarker for efficacy. Robust PCSK9 ELISA methods will however further establish pharmacodynamic target engagement of this new class of lipid lowering therapeutics. These assays may also provide a tool for patient stratification and tailored dosing.

ABBREVIATIONS

PCSK9: proprotein convertase subtilisin kexin type 9, LDLC: low-density lipoprotein-cholesterol, LDLR: low-density lipoprotein receptor, HDLC: high-density lipoprotein-cholesterol, SREBP2: sterol regulatory element binding protein 2, FH: familial hypercholesterolemia, IP: immunoprecipitation, RT-PCR: reverse transcriptase-polymerase chain reaction, HMG-CoA reductase: 3-Hydroxy-3-methyl-glutaryl-CoA reductase, EGF(A): epidermal growth factor like repeat A, FXR: farnesoid X receptor, ASO: antisense oligonucleotide, PPARα: peroxisome proliferator-activated receptor alpha

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