Review Article

A Brief Review on Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9)

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Abstract

Proprotein convertase subtilisin/ kexin type 9 (PCSK9) is a newly identified secreted protein which plays a vital role in cholesterol homeostasis. PCSK9 increases low-density lipoprotein cholesterol (LDL-C) in plasma by inhibiting its clearance via inducing degradation of low-density lipoprotein receptor (LDLR). In humans, PCSK9 level is modulated by factors such as age, gender, gene mutations, hormones, diet, plant products, fasting and diurnal rhythm. Recent research has proposed inhibition of PCSK9 as a beneficial therapeutic approach to control dyslipidemia, importantly in hypercholesterolemia patients. Thus inhibition of PCSK9 appears to be a considerable promising approach to minimize cardiovascular risk these days. In this review, we acknowledge the recent development in the field of PCSK9. We also highlight the reason behind its choice as a novel therapeutic target to combat LDL-C related imbalances in cardiovascular diseases; an area of focus, increasingly explored by researchers in collaboration with pharmaceutical companies.

Key words: Proprotein convertase subtilisin/ kexin type 9 (PCSK9), Low density lipoprotein (LDL), hormones, diet and PCSK9 inhibitor.

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INTRODUCTION

In humans, an increased risk for development of atherosclerosis and cardiovascular events is frequently associated with disturbances in cholesterol metabolism.\(^1\) Cholesterol is a molecule of fundamental importance for normal cell function, and serves as a precursor for steroid hormones and bile acids. The liver has a crucial role in cholesterol homeostasis. In the liver, cholesterol is converted to bile acids that are secreted into bile together with free cholesterol.\(^2\) Circulating lipoproteins provide cells with cholesterol. Cells also acquire cholesterol by de novo synthesis. In blood, plasma cholesterol is transported within lipoproteins such as high-density lipoproteins (HDL), low-density lipoproteins (LDL), very low-density lipoproteins (VLDL) and chylomicrons. Various surface receptors such as low-density lipoprotein receptor (LDLR), scavenger receptor class B type 1 (SRB-1) and very-low-density lipoprotein receptor (VLDLR) are involved in the cellular uptake of lipoproteins.\(^3-5\)

Excessive amounts of plasma cholesterol, especially LDL-cholesterol (LDL-C) are linked to cardiovascular disease.\(^6\) In human blood, 70% of the cholesterol is within LDL particles.\(^7\) Most plasma LDL is taken up by liver while the rest is distributed to peripheral tissues and to the adrenals and gonads.\(^8\) Modulation of hepatic LDL receptors (LDLRs) plays a vital role for LDL-C level in plasma by influencing the clearance of LDL-C from circulation. LDLR numbers are regulated mainly by two mechanisms a) transctional (via the sterol regulatory element-binding protein (SREBP) -2 pathway) \(^9\) and b) post-transcriptional (importantly through the proproteinconvertasesubtilisin/ kexin type 9 (PCSK9) mechanisms.\(^10,11\) The very important role of post-transcriptional regulation by PCSK9 was established during last decade. PCSK9 induces the degradation of hepatic LDLR, thereby elevating cholesterol levels in plasma. Higher PCSK9 levels correlate with cardiovascular complications and thus, inhibiting PCSK9 could be beneficial for minimizing cardiovascular risks in human.

PCSK9

Previously known as neural apoptosis-regulated convertase 1, PCSK9 was discovered in 2003.\(^12,13\) Since then, it has been now well evidenced that PCSK9 plays a vital role in cholesterol metabolism by regulating hepatic LDLR. Though it abundantly expresses in liver, but it is also present to a lesser extent in the small intestine, kidney, and central nervous system. In human, PCSK9 gene is present on chromosome 1p32.3. PCSK9 strongly correlates with cardiovascular complication, including dyslipidemia and atherosclerosis.\(^12-14\) Structurally, alone with a 30 amino acid signal sequence, PCSK9 contains a prodomain (amino acid 31-152), a catalytic domain (amino acids 153-425) and a cysteine histidine rich C-terminal domain (amino acids 426-692).\(^15\) Over expression or gain of function (GOF) mutation of PCSK9 gene leads to hypercholesterolemia whereas, loss of function (LOF) mutations of PCSK9 gene lead to hypocholesterolemia and reduced cardiac risk.\(^16\)

PCSK9 and its role on LDLR

PCSK9, the ninth member of the proproteinconvertase family, is a secreted protein which presumably is secreted from the liver. Within hepatic cells, PCSK9 is synthesized as a precursor (72 Kda) which undergoes autocatalytic cleavage and produces 2 products, the prosegment and the mature PCSK9 (63 KDa).\(^17\) This autocatalytic cleavage is required for maturation of PCSK9 and also for its trafficking from ER to Golgi. Mature PCSK9 is secreted into circulation.\(^16,17\) Before secretion, mature PCSK9 undergoes a number of post-translational modifications such as N-glycosylation, sulphation and phosphorylation.\(^15\) On the cellular membrane, the catalytic domain of circulating PCSK9 binds to the epidermal growth factor precursor homology domain-A (EGF-A) of the LDLR in a calcium-dependent manner.\(^18\) Then the complex of LDLR/PCSK9 is internalized within the cell via clathrin-mediated endocytosis thus entering the endosomal pathway.\(^19\) The bonding affinity of LDLR/PCSK9 complex is increased in the low pH of endosome.\(^20\) As a result of changes in the conformation of LDLR its recycle back to cell membrane is stopped. The complex of LDLR/PCSK9 is then directed to the lysosome and destined for lysosomal degradation.\(^21\) Plasma levels of PCSK9 generally correlate positively with the plasma LDL-C. PCSK9 induces lysosomal LDLR degradation after binding to the LDLR (Figure 1). As a result, it down-regulates cellular LDLR numbers thereby diminishing the cellular
uptake of LDL particles and increasing plasma LDL levels.\textsuperscript{[19-22]} Like secreted PCSK9, intracellular PCSK9 could also lead the lysosomal degradation of LDLR. Within the hepatocyte, before LDLR transport toward to the cellular membrane, the prosegment PCSK9 binds to LDLR and forms the prosegment PCSK9/LDLR complex which is then directed from Golgi to the lysosome for degradation.\textsuperscript{[16,23]} However, the underlying mechanisms behind PCSK9 mediated the degradation of LDLR is not fully understood.

PCSK9 relation to age, gender and other metabolic factors

It is known that plasma cholesterol, especially the levels of LDL-C increases in human with age. In females, this increase of LDL-C is much prominent after menopause. Studies have shown that PCSK9 levels also increase with age in a healthy population, and it is higher in females then in males.\textsuperscript{[24-26]} It is also proposed that PCSK9 is one of the reasons for age-related induction of LDL-C at least in females.\textsuperscript{[25,27]} In addition, PCSK9 has been shown to be positively correlated with LDL-C, total-C, triglyceride, insulin, body mass index (BMI), and glucose with cholesterol synthesis in human.\textsuperscript{[25,26]}

Hormonal effects on PCSK9

Studies have shown that LDL-C metabolism is modulated by hormones such as estrogen (E2), Testosterone (T), adrenocorticotropic hormone (ACTH), thyroid hormone (TH), Growth hormone (GH) and glucagon in human and in animals.\textsuperscript{[25,27-31]} Studies have also shown that hormones such as estrogen, testosterone, thyroid hormone and glucagon can regulate PCSK9 levels transcriptionally or post-transcriptionally in animals, as well as in human.\textsuperscript{[25,27,30,31]}

Estrogen, a female sex hormone has shown to reduce hepatic PCSK9 mRNA and protein levels in animals.\textsuperscript{[31]} Furthermore, in humans, supra-physiological levels of estrogen (prior to in vitro fertilization) reduce plasma PCSK9 and LDL cholesterol levels.\textsuperscript{[27]} However, from post-hoc analysis of postmenopausal females, taking estrogen supplement indicates that exogenous estrogen therapy has failed to reduce circulating PCSK9 in postmenopausal women.\textsuperscript{[26]} It has been reported that PCSK9 and LDL-C may also be regulated during menstruation cycle depending on the estrogen levels in fertile females.\textsuperscript{[25]} However, a study in Chinese population suggests otherwise, where no such correlation was found.\textsuperscript{[32]}

Testosterone, a male sex hormone reduces hepatic PCSK9 mRNA and protein and also circulating PCSK9 in pig\textsuperscript{[33]}, whereas a study in human has shown that circulating PCSK9 is not related to testosterone in males.\textsuperscript{[30]}

Thyroid hormones such as tri-iodo-thyronine (T3) and thyroxine (T4) are synthesized in the thyroid gland. These hormones are proven to be the major regulators of cholesterol homeostasis. Hypothyroidism is strongly associated with enhanced levels of plasma total and LDL cholesterol whereas in hyperthyroidism, it is vice versa. Results indicate that PCSK9 level is lower in patients with hypothyroidism. Treatment with eprotirome, a thyroid hormone supplement reduces plasma PCSK9.\textsuperscript{[28]}

Glucagon and Insulin, two important hormones involved in carbohydrate homeostasis, have also shown to be correlated with PCSK9.\textsuperscript{[25,31]} Glucagon is a peptide hormone produce from alpha cells of the pancreas, increases concentration of glucose in blood whereas insulin, another peptide hormone produced from beta cells of the pancreas regulates carbohydrate metabolism and enhances glucose absorption from blood to muscles.\textsuperscript{[34]} Studies have shown that these two hormones may influence PCSK9 in human.\textsuperscript{[25,31]}

Dietary effects on PCSK9

Dietary nutrition has also shown to regulate PCSK9 in animals and humans. Several studies have found the sterols influences PCSK9 mRNA expressions via SREBP2 pathway.\textsuperscript{[35,36]} Mediterranean diet and diet containing vegetable polyunsaturated fatty acids (PUFAs) reduce circulating PCSK9 in human.\textsuperscript{[37-39]} In humans, it has been also described that high fructose containing diet induces an increase in plasma PCSK9 concentration.\textsuperscript{[40]} PCSK9 is also affected by fasting and follows a diurnal rhythm.\textsuperscript{[41,42]} Additionally, Berberine, an isoquinoline plant alkaloid, found in plants such as Berberis vulgaris, Coptis chinensis, Berberis aristata inhibit hepatic and circulating PCSK9 thereby reducing cardiovascular risk.\textsuperscript{[43,44]}

PCSK9 as a therapeutic target
Figure 1: A) Degradation of LDLR and involvement of PCSK9; B) LDL-C clearance and recycling of LDLR.

Abbreviations: Low density lipoprotein cholesterol- (LDL-C); LDL receptors- (LDLRs); Proproteinconvertasesubtilisin/kexin type 9- (PCSK9).

Table 1: Pharmaceutical advancement in development of PCSK9 inhibitors

<table>
<thead>
<tr>
<th>Treatment approaches to inhibit PCSK9</th>
<th>Pharmaceutical companies</th>
<th>Present status and development</th>
</tr>
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<tbody>
<tr>
<td>Monoclonal antibodies</td>
<td>Sanofi/Regeneron</td>
<td>FDA approved, July 2015</td>
</tr>
<tr>
<td></td>
<td>Amgen</td>
<td>FDA approved, August 2015</td>
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<tr>
<td></td>
<td>Pfizer/Genetech</td>
<td>Phase III ongoing</td>
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<tr>
<td></td>
<td>Eli Lilly</td>
<td>Phase II completed</td>
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<td></td>
<td>Novartis</td>
<td>Phase I completed</td>
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<tr>
<td>siRNA</td>
<td>Alnylam Pharmaceuticals</td>
<td>Phase I completed</td>
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<tr>
<td></td>
<td></td>
<td>Phase I recruiting</td>
</tr>
<tr>
<td>Mimetic peptides</td>
<td>Serometrix</td>
<td>Preclinical</td>
</tr>
<tr>
<td></td>
<td>Merck &amp; Co</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Adnectin</td>
<td>BMS-Adnexus</td>
<td>Phase I completed</td>
</tr>
<tr>
<td>Small molecules</td>
<td>Shifa Biomedical Corp</td>
<td>Preclinical completed</td>
</tr>
</tbody>
</table>

Abbreviations: Proproteinconvertasesubtilisin/kexin type 9- (PCSK9); Food and drug administration (FDA).
Inhibition of circulating and intercellular PCSK9 is a novel therapeutic approach for the treatment of dyslipidemia.\textsuperscript{15,17,24} As discussed earlier, loss of function (LOF) mutations of PCSK9 gene reduces the cardiac risk by decreasing cholesterol levels.\textsuperscript{45} Moreover, most commonly used lipid lowering drugs such as statins, the inhibitor of cholesterol biosynthesis via SREBP2 pathway, and Ezetimibe, the only cholesterol absorption inhibitor, reported to induce PCSK9 in human, motivated the need for development of PCSK9 inhibitors in the pharmaceutical market. Interestingly, the approach of PCSK9 inhibition either by monoclonal antibody or via some other approach such as mimetic peptides, adnectin, siRNA and small molecules turns out to be a promising treatment for cardiovascular disease.\textsuperscript{46} These molecules are undergoing clinical trials in different phases (Table 1).\textsuperscript{46}

**SUMMARY**

Results and progress of different clinical trials, as mentioned in table 1, indicate that PCSK9 inhibitors alone or combination with other lipid lowering drugs could be a successful treatment for hypercholesterolemia in near future.

**DISCLOSURES**

There is no conflict to disclose.

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