UNDERGRADUATE RESEARCH IN NATURAL AND CLINICAL SCIENCE AND TECHNOLOGY (URNCST) JOURNAL
Read more URNCST Journal articles and submit your own today at: https://www.urncst.com

RESEARCH PROTOCOL OPEN ACCESS

Investigating SHP and PCSK9 Interactions in Cholesterol-Mediated Cardiovascular Diseases: A Research Protocol

Moon Young Bae, BHSc (Honours) Student [1]*, Rachel Kim, BHSc (Honours) Student [2], Judy Wang, HBSc Student [3]

[1] Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 1N4
[2] Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada KNL 3N6
[3] Department of Immunology and Human Biology, University of Toronto, Toronto, Ontario, Canada M5S 1A1

*Corresponding Authors: moonyoung.bae@ucalgary.ca

Abstract

Introduction: Improper cholesterol metabolism results in accumulation of low-density lipoproteins (LDL). High levels of LDL cholesterol deposits in blood vessels, forming plaques and contributing to various cardiovascular diseases (CVD). The nuclear farnesoid X receptor (FXR) regulates the transcription of genes involved in cholesterol metabolism and is a therapeutic target for cholesterol dysregulation. Studies conducted on immortalized human hepatocytes demonstrate FXR signaling-induced downregulation of proprotein convertase subtilisin/kexin type 9 (PCSK9) expression. PCSK9 is an LDL receptor-degrading enzyme whose upregulation is implicated in cholesterol-mediated diseases. Specifically, the FXR target gene SHP (small heterodimer partner) is a transcriptional regulator that has been implicated in an inverse relationship with PCSK9 expression. The biomolecular mechanism mediating this relationship has not been explored, meriting investigation into a potential novel axis of cholesterol metabolism. We hypothesize that SHP is a direct repressor of PCSK9 transcription.
Methods: To investigate, we will knock out SHP expression in the liver hepatocyte cell line AML12 using small interfering RNAs (siRNAs). To confirm SHP knockout on transcriptomic and proteomic levels, reverse transcription quantitative PCR (RT-qPCR) and Western blotting will be performed. To assess SHP binding to the promoter region of PCSK9, an electrophoretic mobility supershift (EMSA) assay will be performed on unstimulated or chenodeoxycolic acid (CDCA)-stimulated AML12 cells that have undergone SHP or control knockouts. Western blotting will quantitate PCSK9 protein expression following SHP knockout in CDCA-stimulated and unstimulated conditions.
Results: Results from EMSA are expected to demonstrate SHP binding to the promoter region of PCSK9 in a transcription factor complex to repress transcription. SHP knockout models are expected to show upregulated PCSK9 expression at transcriptomic and proteomic levels.
Discussion: If successful, our study presents a novel perspective on cholesterol metabolism by characterizing the inhibitory effect of SHP on PCSK9 expression. This underlines the critical role of FXR signaling in PCSK9 regulation, and knockout models and assay techniques provide valuable evidence of this regulatory role.
Conclusion: This study will establish an enhanced understanding of the SHP/PCSK9 pathway within broader pathways of cholesterol metabolism. Further research may explore therapies targeting the SHP/PCSK9 pathway to manage CVD downstream of cholesterol dysregulation.

Keywords: SHP; PCSK9; FXR; cholesterol metabolism; cardiovascular diseases; transcription regulation; gene expression

Introduction

Serum cholesterol levels have consistently been implicated in a wide range of CVD pathophysiologies, including those of atherosclerosis [1], hypertensive heart disease (HHD), ischemic heart disease (IHD), and stroke [2,3]. In addition to a significantly increased risk of mortality, raised total serum cholesterol accounts for approximately 29.7 million Disability-Adjusted Life Years (DALYs) according to the WHO, corresponding to a significant global burden of disease (GBD) [4].

In particular, blood composition of various cholesterol-carrying lipoproteins has been linked to cholesterol-related CVD. Lipoproteins are aggregations of proteins and lipids such as triacylglycerols and cholesteryl esters functioning to transport lipids throughout the body. Low-density lipoprotein (LDL) and high-density lipoprotein (HDL), with high and low lipid content respectively, are responsible for the transport of cholesterol and cholesteryl esters from the

liver and diet through the circulation and to and from body tissues. LDL is produced in the liver and functions to transport cholesterol to body tissues, where receptor proteins called LDL receptors (LDLR) bind to and extract cholesterol from LDL [5]. In contrast, HDL functions to recover fragments of cholesteryl esters and unesterified cholesterol from the walls of blood vessels, thus preventing the buildup of fatty plaques. As a result, HDL is considered a protective factor against the development of cholesterol-related CVD [1,6,7]. Moreover, raised serum levels of low-density lipoprotein (LDL) and/or reduced levels of high-density lipoprotein (HDL) are associated with greater risks of CVD.

Several key players of interest within cholesterol metabolism pathways include the farnesoid X receptor (FXR), which is responsible for regulating the transcription of genes involved in cholesterol metabolism [8]. Moreover, it has been proposed as a potential target for atherosclerosis and hyperlipidemia treatment, indicating its key metabolic role [8]. Specifically, FXR regulates the expression of pro-protein convertase subtilisin/kexin 9 (PCSK9) and the transcription factor small heterodimer partner nuclear receptor (SHP) [8,9]. SHP is known to act by binding to a transcription factor complex, which notably includes sterol regulatory element-binding protein 2 (SREBP-2), and its upregulation has been shown to promote cholesterol absorption [10-12]. SREBP-2 is a major transcription factor for which SHP has been implicated as a global binding partner, especially in the regulation of pro-protein convertase subtilisin/kexin 9 (PCSK9) expression [10,11]. PCSK9 is an enzyme that degrades LDLR [8,9].Further research demonstrates that a deficit of LDLR reduces LDL binding and thus increases plasma LDL; therefore, PCSK9 regulation is crucial to maintaining healthy blood lipoprotein ratios [13].

Current research and literature indicate an inverse relationship between SHP and PCSK9 expression through analysis of mRNA levels [9]; however, neither a causal relationship nor an exact mechanism for this interaction has been identified. Considering the crucial role of serum cholesterol and the metabolism thereof, we aim to establish a clearer understanding of the nature of the SHP/PCSK9 relationship and to further explore its implications for CVD. In lieu of the finding that SHP is a global binding partner of SREBP-2, an explanatory mechanism for the inverse relationship between SHP and PCSK9 is possible. Thus, we hypothesize that SHP acts to repress PCSK9 transcription through binding in a transcription factor complex to SREBP-2.

Methods

Cell Culture

Immortalized murine AML12 liver hepatocytes (American Type Culture Collection (ATCC)) were cultured in Dulbecco's Modified Eagle Medium with F12 (DMEM:F12) containing 10% heat-inactivated fetal bovine serum (FBS), 10 ug/mL insulin, 5.5 ug/mL transferrin, 5 ng/mL selenium and 40 ng/mL dexamethasone following ATCC recommendations. Cells were incubated in a 5% CO_2 incubator at 37°C in adherent T75s flasks.

SHP Knockout

AML12 liver hepatocytes were plated in 6-well plates at 5.0 x 10^5 cells/mL and allowed to adhere, recover, and grow before transfection. Cells were plated in normal culture medium without FBS. The cells were then forward transfected 24 hours after plating with anti-SHP siRNA, scramble siRNA or anti-GAPDH siRNA or were left untransfected. Opti-MEM™ Reduced Serum Medium was used to dilute Lipofectamine 2000 reagent, siRNAs and plasmid DNA for transfection. 0.6 ug of siRNA was added per 1 ug of plasmid DNA and incubated with Lipofectamine™ 2000 at a 1:1 ratio following Invitrogen protocol. Cells were cultured with Lipofectamine™ 2000 complexes for 48 hours in a 37°C 5% CO_2 incubator before use in experiments.

Design of siRNAs

Anti-SHP (Assay 102839), scramble *Silencer*™ negative control (Catalog #AM4311) and anti-GAPDH siRNAs (Assay 505463) were purchased from Thermo Fisher Scientific.

Chemicals

Chenodeoxycholic acid (CDCA) is a major component of bile acid and a potent endogenous FXR ligand. It was used to induce FXR stimulation and transcription of target genes, including SHP. CDCA was purchased from Cayman Chemical (Item no. 1001128). AML12 cells were cocultured with CDCA for 48 hours at a concentration of 50 uM as previously described in Langhi et al. [9].

Western Blotting

Protein expression and quantity was analyzed by Western blot, where cells were lysed via incubation with radioimmunoprecipitation assay (RIPA) buffer supplemented with a protease inhibitor cocktail (Roche) and microcentrifuged at 12,000 rpm for 20 minutes. A Bradford assay was then performed to determine the appropriate protein concentration. 20 ug of each protein lysate was then resolved on SDS-PAGE gels before transfer onto a nitrocellulose membrane. Membranes were blocked using 5% non-fat dry milk and incubated with anti-SHP1 monoclonal antibody (Thermo Fisher Catalog #MA5-14839) at a 1:500 dilution, anti-PCSK9 polyclonal antibody (Thermo Fisher Catalog #55206-1-AP) at a 1:1000 dilution or anti-β actin recombinant monoclonal antibody (Thermo Fisher Catalog #MA5-32479) at a 1:10,000 dilution for one hour at room temperature. β actin acted as a loading control. Bound proteins were then detected using a secondary horseradish peroxidase-conjugated goat anti-rabbit antibody (Thermo Fisher Catalog #31460) and the

eBioscience™ DAB Advanced Chromogenic Kit (Invitrogen).

Electrophoresis Mobility Supershift Assay (EMSA)

EMSA was used to evaluate the state of SHP binding to the promoter of *PCSK9*. CDCA was used to stimulate SHP-KO and wild type AML12 liver hepatocytes, for comparison with unstimulated SHP-KO and wild type AML12 liver hepatocytes. Nuclear lysate was extracted from cells using the protocol described by Luo et al. [14] Simultaneously, single-stranded DNA probes specific for the *PCSK9* promoter Jeong et al. [15] were labeled on the 3' end with biotin-11-UTP using the Pierce™ Biotin 3' End DNA Labeling Kit. Nuclear proteins were pre-incubated with anti-SHP1 polyclonal antibody (Thermo Fisher Catalog #PA5-118222) at a 1:500 dilution and anti-SREBP-2 polyclonal antibody (Thermo Fisher Catalog #SREBF-201AP) at a 1:250 dilution for 30 minutes before addition of labeled DNA probes following previous protocols [16,17]. Electrophoresis was run on a 6% native polyacrylamide gel in Tris-borate-EDTA running buffer (TBE buffer) at 4°C for 1.5 hours. Gels were then visualized using the LightShift™ Chemiluminescent EMSA Kit. A competitive EMSA was performed alongside to evaluate the specificity of protein binding to DNA. Unlabeled DNA probes were added in excess to nuclear protein extract and pre-incubated for 10 minutes before the addition of the biotin-labeled probe.

Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR)

RT-qPCR was used to assess mRNA levels of *PCSK9* following siRNA knockout of SHP and to confirm SHP KO by siRNA. Total RNA content was isolated from CDCA-stimulated wild type, anti-SHP siRNA-treated, scramble siRNA-treated or unstimulated anti-SHP siRNA-treated AML12 liver hepatocytes using the TRIzol™ Plus RNA Purification Kit (Invitrogen Catalog #12183555) supplemented with RNase-free DNase I (Qiagen). RT-qPCR was then performed following the protocol described in Costet et al. with the following primers, also described in Costet et al. [18] and Langhi et al. [9]:

Mouse SHP:
5'-CTCTTCCTGCTTGGGTTGGC forward,
5'-GCACATCGGGGTTGAAGAGG reverse

Mouse 18S (housekeeper):
5′-GCACATCGGGGTTGAAGAGG forward,
5′-AAACTCTGGTGGAGGTCCGT reverse

Along with RT-qPCR of experimental conditions, a condition including all components except for reverse transcriptase was used as control to check for DNA contamination.

Results

We predict that SHP is a regulator of *PCSK9* expression through exhibiting an inhibitory effect on the expression of *PCSK9*. This is potentially achieved through interaction with other transcription factors, like SREBP-2. It is expected that EMSA will demonstrate SHP binding to the promoter region of *PCSK9* via impeding DNA movement down the gel, and anti-SHP antibody binding will confirm SHP presence at the promoter. This will be indicated via hyper-impediment of DNA movement. Quantification of gene expression by RT-qPCR, corroborated by markers of PCSK9 found via Western blot in SHP-KO AML12 cells, is expected to reflect the relative level of SHP binding. Low SHP binding resulting from SHP KO is expected to correlate with high levels of PCSK9 and the SHP KO model is expected to demonstrate upregulated expression of PCSK9 at both mRNA and protein levels due to the absence of inhibitory regulation by SHP. No promoter binding by SHP is expected by EMSA analysis in this knockout model, and a thick, dark band is expected on the Western blot when stained for PCSK9.

Discussion

The objective of this study is to examine the relationship of SHP in the regulation of *PCSK9* gene expression. With the anticipation that SHP functions as a critical modulator of *PCSK9* expression, we aim to shed light on the underlying mechanisms influencing cholesterol metabolism.

Should the findings be consistent with our expected results, it would support the hypothesis that SHP exerts a direct inhibitory effect on *PCSK9* transcription. The results could be interpreted to suggest important consequences of SHP deficiency on LDL cholesterol clearance, which would warrant further study. This is because PCSK9 is known for its role in promoting the degradation of LDLR, thereby reducing the uptake of LDL cholesterol from the bloodstream [1]. If there is increased PCSK9 activity in the SHP KO cells, it is expected that there would be decreased availability of functional LDLR. This, in turn, would result in elevated circulating LDL cholesterol levels. The results of the RT-qPCR and EMSA would also allow for quantitative assessment of changes in *PCSK9* mRNA levels in SHP KO cells, which could also be used to verify the successful knockout of SHP by siRNA, ensuring the specificity and effectiveness of this experimental approach. Should results be as expected, this would serve as a reliable measure of the regulatory effect of SHP on *PCSK9* expression.

If our results show that SHP binds to the *PCSK9* promoter, it would provide evidence of a direct mechanism by which FXR signaling modulates *PCSK9* expression through the regulation of SHP. Specifically, the actions of SHP would highlight the role of FXR as a master regulator

of PCSK9 expression through an SHP-mediated intermediary role in transcriptional repression [20].

Our initial hypothesis suggests direct binding of SHP to the promoter site of the *PCSK9* gene. However, in the case where our results do not indicate such binding, the presence of SHP alongside other transcription factors is a viable alternative explanation. Current literature suggests that SHP is often found in complex with other transcriptional factors, such as SREBP-2, to perform its function as a transcriptional inhibitor of gene expression [19].Thus, it may be possible that SHP binds to a transcription factor complex to facilitate a more intricate regulatory network for *PCSK9* transcription, rather than acting in isolation. This would explain results that demonstrate a lack of direct SHP binding to the promoter region, as other transcription factors would not be present *in vitro*. In this case, further exploration of these potential interactions between SHP and other transcription factors could unveil a more comprehensive understanding of the complex regulatory mechanisms that govern *PCSK9* expression. Such insights into these relationships may provide valuable clues for future *in vivo* or *in vitro* studies to explore specific implications and effects on CVD Modulating SHP activity is of interest in implicating CVD, because of its role in repressing PCSK9. When PCSK9 is overactive, it leads to a reduces number of LDL receptors, resulting in elevated LDL cholesterol in the blood. This is a significant risk factor for CVD such as atherosclerosis and hypercholesterolemia. Further, understanding this relationship may help contribute to the development of diagnostic tests associated with CVD.

Strengths and Limitations

This proposed study exhibits several notable strengths that enhance the significance of the expected research findings. Firstly, it adopts a novel approach in investigating the interplay between SHP and PCSK9, allowing for the examination of their regulatory roles in lipid metabolism. By delving into this intricate relationship, the study contributes to advancing our understanding of the complex mechanisms involved in cholesterol metabolism. Moreover, the research employs multiple methods, including EMSA, Western blot, and RT-qPCR analysis to validate and strengthen the reliability of the expected results. The integration of these complementary techniques ensures a robust examination of SHP's role in modulating *PCSK9* expression. Further, the use of SHP KO cells as an *in vitro* model allows for a detailed examination of the molecular mechanisms involved in SHP-mediated PCSK9 regulation.

The study acknowledges certain limitations that should be considered while interpreting the results. Since the study was conducted in AML12 cells of murine origin, results may not be directly generalizable to human metabolism. Further, the use of immortalized cells may pose a limitation, as these cells have been altered to prevent metabolic and reproductive senescence. An *in vitro* design also limits generalizability of the results since various environmental factors could influence the interplay between SHP and PCSK9.

Conclusions

Cholesterol-mediated diseases pose a significant mortality risk and disease burden worldwide. The presence of the LDLR-degrading enzyme, PCSK9, is a well-characterized prognostic factor and player driving negative cardiovascular outcomes and more severe manifestations of disease. Thus, understanding the associated pathways of PCSK9 regulation is paramount. Through the FXR-mediated axis of cholesterol metabolism, the involvement of SHP in directly repressing *PCSK9* expression has been previously implied. Further research has demonstrated the direct interaction of SHP with SREBP-2, a major transcriptional activator of *PCSK9* expression. Thus, this relationship could play a significant role in modulating CVD risk in individuals. Our study aims to explore the nature of the interaction between SHP, *PCSK9* and their gene products in the context of cholesterol metabolism, with the expectation that SHP will bind to the promoter region of *PCSK9* in an inhibitory manner to repress transcription. We also expect levels of PCSK9 to reflect SHP levels in an inverse manner; thus, we expect SHP knockout models to upregulate PCSK9 expression on both mRNA and protein levels. Assuming EMSA demonstrates SHP binding to the promoter region of PCSK9, further research may be conducted to determine how this interaction can be targeted to develop novel treatments for cholesterol-related diseases. This includes conducting *in vivo* animal studies and studies in primary human hepatocytes, which will be crucial in validating the relevance of our expected findings. Moreover, results may be applied to genetic studies to identify individuals who are genetically at risk for altered SHP expression, which may predispose them to CVD via disruptions in the SHP/PCSK9 pathway. Another application may concern the design of novel therapeutic interventions targeting this pathway. For example, a drug that mimics SHP action could potentially reduce PCSK9 activity and enhancing the clearance of LDL cholesterol. Overall, understanding the relationship between SHP and *PCSK9* would allow for further research to draw direct links to and explore the effects on diseases in which cholesterol dysregulation is a factor in its pathogenesis.

List of Abbreviations Used

LDL: low-density lipoprotein
FXR: farnesoid X receptor
SREBP-2: sterol regulatory element binding protein 2
siRNA: small interfering RNA
RT-qPCR: reverse transcription quantitative polymerase chain reaction
EMSA: electrophoretic mobility supershift assay
CVD: cardiovascular disease(s)

HHD: hypertensive heart disease
IHD: ischemic heart disease
DALY(s): disability-adjusted life year(s)
GBD: global burden of disease
HDL: high-density lipoprotein
LDLR: low-density lipoprotein receptor
SHP: small heterodimer partner
PCSK9: pro-protein convertase subtilisin/kexin 9
KO: knockout
DMEM:F12: Dulbecco's modified eagle medium with F12
CDCA: chenodeoxycholic acid
RIPA: radioimmunoprecipitation assay

Conflicts of Interest
The author declares that they have no conflict of interests.

Ethics Approval and/or Participant Consent
This study comprises only a research proposal for a research protocol. Thus, no participants were recruited for the completion of the study, nor was ethical approval attained. When carrying out the study, researchers should obtain and adhere in full to animal experimentation ethics and guidelines.

Authors' Contributions
MYB: ideation, design of the study, collected and analyzed literature and data, drafted and revised the manuscript, and gave final approval of the version to be published.
RK: study design, ideation, collected and analyzed data and existing literature, drafted and revised the manuscript, and gave final approval of the version to be published.
JW: conceptualization and ideation of the study design, collected and analyzed data and current literature, drafted and revised the manuscript, and gave final approval of the version to be published.

Acknowledgements
The authors would first like to extend our thanks to the Undergraduate Research in Natural and Clinical Sciences and Technology Journal. Secondly, we thank our mentor, Alexandra Akman, for her guidance and encouragement throughout the development of this proposal. Finally, we thank all executive members, sponsors, and partners of the IgNITE Medical Case Competition for this chance to extend our understanding of cardiorespiratory research.

Funding
This study was not funded.

References
[1] Wang HH, Garruti G, Liu M, Portincasa P, Wang DQH. Cholesterol and lipoprotein metabolism and atherosclerosis: Recent advances in reverse cholesterol transport. Ann Hepatol. 2017 Nov;16(Suppl. 1):s27–42. https://doi.org/10.5604/01.3001.0010.5495
[2] Yusuf S, Hawken S, Ôunpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): A case-control study. Lancet. 2004 Sept 11;364(9438):937–52. https://doi.org/10.1016/S0140-6736(04)17018-9
[3] Jung E, Kong SY, Ro YS, Ryu HH, Shin SD. Serum cholesterol levels and risk of cardiovascular death: A systematic review and a dose-response meta-analysis of prospective cohort studies. Int J Environ Res Public Health. 2022 Jul;19(14)8272. https://doi.org/10.3390/ijerph19148272
[4] The Global Health Observatory. Indicator metadata registry list: Raised cholesterol. [Internet]. World Health Organization. Available from: https://www.who.int/data/gho/indicator-metadata-registry/imr-details/3236
[5] Rinninger F, Heine M, Singaraja R, Hayden M, Brundert M, Ramakrishnan R, et al. High density lipoprotein metabolism in low density lipoprotein receptor-deficient mice. J Lipid Res. 2014 Sept;55(9):1914–24. https://doi.org/10.1194/jlr.M048819
[6] Carson JAS, Lichtenstein AH, Anderson CAM, Appel LJ, Kris-Etherton PM, Meyer KA, et al. Dietary cholesterol and cardiovascular risk: A science advisory from the American Heart Association. Circulation. 2020 Jan 21;141(3):e39–53. https://doi.org/10.1161/CIR.0000000000000743
[7] Casula M, Colpani O, Xie S, Catapano AL, Baragetti A. HDL in atherosclerotic cardiovascular disease: In search of a role. Cells. 2021 Aug;10(8):1869. https://doi.org/10.3390/cells10081869
[8] Mencarelli A, Fiorucci S. FXR an emerging therapeutic target for the treatment of atherosclerosis. J Cell Mol Med. 2010 Mar 14;14(1-2):79–92. https://doi.org/10.1111/j.1582-4934.2009.00997.x
[9] Langhi C, Le May C, Kourimate S, Caron S, Staels B, Krempf M, et al. Activation of the farnesoid X receptor represses PCSK9 expression in human hepatocytes. FEBS Lett. 2008 Feb 25;582(6):949–55. https://doi.org/10.1016/j.febslet.2008.02.038
[10] Kim Y, Byun S, Zhang Y, Seok S, Kemper B, Ma J, et al. Liver ChIP-seq analysis in FGF19-treated mice reveals SHP as a global transcriptional partner of SREBP-2. Genome Biol. 2015 Dec;16:268. https://doi.org/10.1186/s13059-015-0835-6
[11] Dong B, Wu M, Li H, Kraemer FB, Adeli K, Seidah NG, et al. Strong induction of PCSK9 gene expression through HNF1alpha and SREBP-2: mechanism for the resistance to LDL-cholesterol lowering effect of statins in dyslipidemic hamsters. J Lipid Res. 2010 Jun;51(6):1486-1495. https://doi.org/10.1194/jlr.M003566

[12] Kerr TA, Saeki S, Schneider M, Schaefer K, Berdy S, Redder T, et al. Loss of nuclear receptor SHP impairs but does not eliminate negative feedback regulation of bile acid synthesis. Dev Cell. 2002 Jun;2(6):713–20. https://doi.org/10.1016/S1534-5807(02)00154-5

[13] Pirahanchi Y, Sinawe H, Dimri M. Biochemistry, LDL cholesterol. [Internet]. StatPearls; 2022 [cited 2023 Aug 5]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK519561/

[14] Luo Y, Hara T, Ishido Y, Yoshihara A, Oda K, Makino M, et al. Rapid preparation of high-purity nuclear proteins from a small number of cultured cells for use in electrophoretic mobility shift assays. BMC Immunol. 2014 Dec;15:586. https://doi.org/10.1186/s12865-014-0062-z

[15] Jeong H, Lee H, Kim K, Kim Y, Yoon D, Park S. Sterol-dependent regulation of proprotein convertase subtilisin/kexin type 9 expression by sterol-regulatory element binding protein-2. J Lipid Res. 2008 Feb;49(2):399–409. https://doi.org/10.1194/jlr.M700443-JLR200

[16] Nakase K, Cheng J, Zhu Q, Marasco W. Mechanisms of SHP-1 P2 promoter regulation in hematopoietic cells and its silencing in HTLV-1-transformed T cells. J Leukoc Biol. 2009 Jan;85(1):165–74. https://doi.org/10.1189/jlb.0608383

[17] Li H, Dong B, Park S, Lee H, Chen W, Liu J. Hepatocyte nuclear factor 1α plays a critical role in PCSK9 gene transcription and regulation by the natural hypocholesterolemic compound berberine. J Biol Chem. 2009 Oct;284(42):28885–95. https://doi.org/10.1074/jbc.M109.052407

[18] Costet P, Cariou B, Lambert G, Lalanne F, Lardeux B, Jarnoux AL, et al. Hepatic PCSK9 expression is regulated by nutritional status via insulin and sterol regulatory element-binding protein 1c*. J Biol Chem. 2006 Mar;281(10):6211–8. https://doi.org/10.1074/jbc.M508582200

[19] Zhang Y, Hagedorn CH, Wang L. Role of nuclear receptor SHP in metabolism and cancer. Biochim Biophys Acta. 2010 Oct 20;1812(8):893–908. https://doi.org/10.1016/j.bbadis.2010.10.006

[20] Jiao Y, Lu Y, Li X. Farnesoid X receptor: A master regulator of hepatic triglyceride and glucose homeostasis. Acta Pharmacol Sin. 2015 Jan;36(1):44–50. https://doi.org/10.1038/aps.2014.116

Article Information
Managing Editor: Jeremy Y. Ng
Peer Reviewers: Alexandra Akman, Clara Rose Schott, Miriam Basta
Article Dates: Received Aug 07 23; Accepted Nov 07 23; Published Dec 29 23

Citation
Please cite this article as follows:
Bae MY, Kim R, Wang J. Investigating SHP and PCSK9 interactions in cholesterol-mediated cardiovascular diseases: A research protocol. URNCST Journal. 2023 Dec 29: 7(12). https://urncst.com/index.php/urncst/article/view/530
DOI Link: https://doi.org/10.26685/urncst.530