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The role of the PCSK9 gene in atherosclerotic plaque based on public database and experimental verification

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Abstract: Objective: PCSK9 is a protein-coding gene which has been implicated in the formation of atherosclerotic plaques in related studies. Nevertheless, there is limited research on the manifestation of it in atherosclerosis and the underlying mechanisms. Data mining and experimental verification were used to ascertain the expression level of this study in the atherosclerosis rat model.

1. Methods

Profile data of PCSK9 genes differentially expressed in atherosclerosis were obtained from the mRNA microarray data set in GEO database, and statistical analysis was conducted to evaluate the results. Subsequently, a model of atherosclerosis was constructed. They were randomly divided into a control group, a model group, a shRNA (short hairpin RNA) - NC interference model group, and a Pcsk9 shRNA interference model group. The expression level of PCSK9 protein in atherosclerotic plaque was further ascertained by combining the results of western blot.

Result The data set from GEO database was statistically analyzed and it was found that the gene was significantly upregulated in the atherosclerotic group ($p < 0.05$). Western blot was employed to further validate the expression level of PCSK9 protein in atherosclerotic plaque tissues in comparison to normal tissues.

Conclusion: PCSK9 gene expression in atherosclerotic plaque is higher than that in normal tissue, indicating that the gene may be implicated in the formation and progression of atherosclerosis.

2. Introduction

The target of PCSK9 offers a fresh perspective for investigating the cause and progression of atherosclerotic plaque.

Atherosclerosis (AS) is a major contributor to cardiovascular disease, and is identifiable by the alteration of the walls of the middle and large arteries [1]. It is widely accepted that inflammation and

abnormal lipid metabolism are two of the theories that explain the development of AS [2], although the precise cause remains undetermined [3]. At the same time, a large number of cardiovascular events are caused by plaque rupture after atherosclerosis [4]. Investigating the manifestation and progression of plaque, in addition to seeking effective treatment strategies and the multi-target action process of existing successful drugs, has become a widely discussed issue in the field of cardiovascular medicine both domestically and internationally.

The ninth member of the Kexin-like proinvertase Bacillus subtilis protease family (PCSK9) is a member of the proprotein converting enzyme subtilisin [5]. Invertase 1 protein encodes neuronal apoptosis [6]. PCSK9 inhibition has been proven to reduce plasma LDL-C levels in both healthy individuals and those with high cholesterol who are taking statins, thus decreasing the risk of cardiovascular disease-related morbidity and mortality[7]. Despite the absence of research on the PCSK9 gene and AS plaques, the relationship between them is still yet to be established.

Data mining is a form of scientific research [8], which focuses on the development of computer models to predict biological results based on the input biological data. Consequently, we forecasted the circRNA-binding miRNA and its target gene [9], and examined the expression of PCSK9 gene in atherosclerotic diseases. It is clear that the connection between the prognosis and the gene is not particularly strong. The purpose of this study is to verify the relationship between the PCSK9 gene and atherosclerotic plaque by using a mouse atherosclerosis model.

3. Materials and methods

3.1. Bioinformatics screening

3.1.1 Data collection

Data sets selected for download from the GEO database, miRNA expression profile GSE100927 based on the GPL17077 ([miRNA-3] Affymetrix Multispecies miRNA-3 Array) platform, were standardized and homogenized.

3.1.2 Screening of differentially expressed miRNAs and hierarchical clustering analysis.

GEO2R (https://www.ncbi.nlm.nih.gov/geo/ge2r) is an online data analysis tool that can be utilized to examine GEO data series gathered from the same experimental conditions. In this study, GEO2R was used to screen differentially expressed miRNAs between atherosclerotic and non-atherosclerotic patients.

3.1.3 Ascertain the level of gene expression.

RSEM software is utilized to measure the expression level of the identified genes, and a block diagram is created to compare the gene expression levels between different groups. Principal component analysis was performed with R-packet gModels (http://www.r-project.org/)). The EDGER package (http://www.r-project.org/) can be used to identify the differentially expressed genes in a sample or group.

3.2 Animal model grouping, establishment and intervention

3.2.1 Experimental animals

Forty SPF-grade, eight-week-old male SD rats, with a mass of 160~200 g, have passed the relevant experimental animal ethics.

3.2.2 Main reagents and instruments

Short Hairpin RNA (shRNA) and shRNA-negative control (NC) recombinant lentiviral plasmids, lentiviral packaging plasmids PGAG/POL, PREV, and PVSV-G (from Shanghai Gima Genetics Company); and RNAPURIFICATIONSYSTEM (from Qiagen, Germany).

3.2.3 Construction of an Atherosclerotic Mouse Model

All rats established an atherosclerotic model after one week of adaptive feeding. The rats were randomly divided into control group, model (hyperlipidemia) group, shRNA-NC interference model (hyperlipidemia + shRNA-NC) group and Pcsk9-shRNA interference model (hyperlipidemia + Pcsk9-shRNA) group with 10 rats in each group. For the purpose of establishing an atherosclerotic model, all rats were provided with food for an additional 12 weeks. The rats in the control group were fed with a standard experimental feed and intraperitoneally injected with saline 2m/kg d for the first three days. After 12 weeks, 1 ml per kg body weight was injected intravenously in a normal saline. In the model group, the rats were given a cholesterol diet which consisted of a normal laboratory diet plus 1% cholesterol and 0.5% cholic acid. During the experiment, shRNA-NC interference model group and Pcsk9-shRNA interference model group were injected intraperitoneally with lentivirus vector shRNA-NC and Pcsk9shRNA, respectively, at a dose of 1 ×108TU/mL per rat every 10 days.

3.3 Detection of the PCSK9 gene in atherosclerotic mice and a control group

Proteins in aortic tissue were separated by protein blotting using 10% SDS-PAGE and then transferred to a PVDF membrane. 5% skim milk was used to block the membrane, and the proteins were incubated with a primary antibody overnight at 4℃. The primary antibodies used were those specific to PCSK9 and GAPDH. PBS was washed, followed by a 2-hour incubation with goat anti-rabbit IgG-HRP secondary antibody. Utilizing enhanced chemiluminescence, a color development technique was employed to evaluate the levels of protein expression.

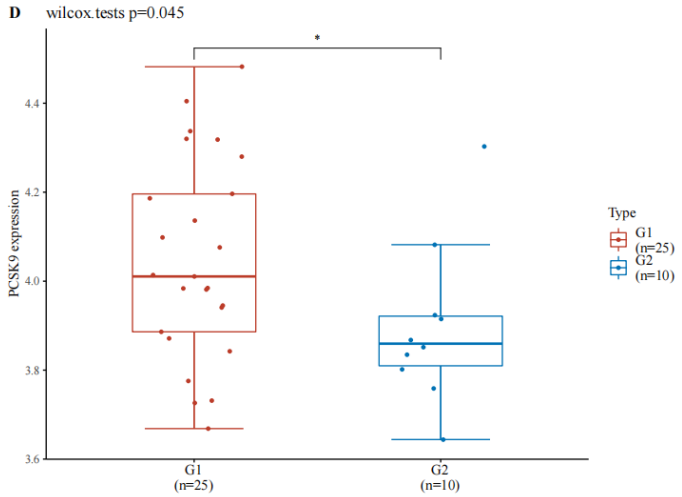
3.4 Statistical analysis uses statistical software SPSS 20.0 for analysis.

Quantitative data that follows the normal distribution is represented by X ± S, and One-Way ANOVA is utilized to compare multiple groups. Wilcox is then used to assess the significance of the two groups. $P<0.05$ indicates that the difference is statistically significant.

4. Results

4.1 Bioinformatics Screening Results

Through the combination of miRNA and mRNA array analysis, we investigated two groups of control arteries, one with and one without atherosclerotic lesions, in a study. Following the collection of RNA, the utilization of microarray and the analysis of bioinformatics data, the disparity in the expression of the PCSK9 gene between two groups of samples can be observed. Data analysis revealed that the gene was significantly overexpressed in the atherosclerotic group, with a p-value of below 0.05, thus suggesting that the gene may be implicated in the development of atherosclerosis. As shown in Figure 1, there is a discrepancy in gene expression between the two groups.



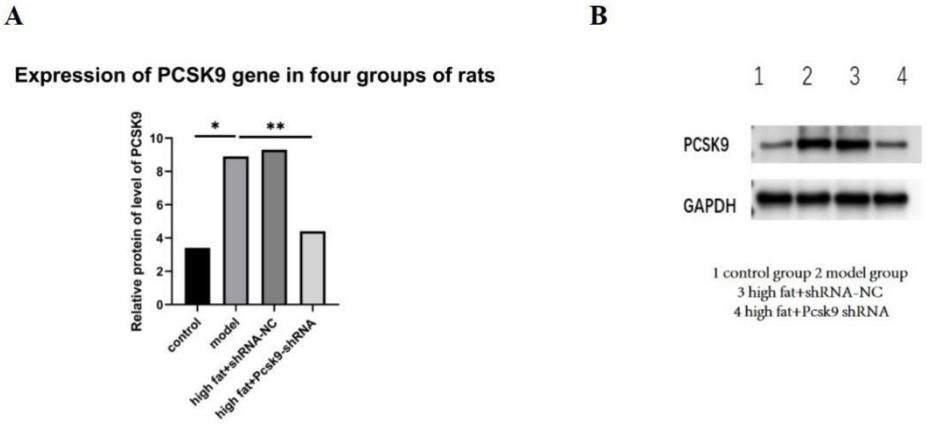
Distribution of the PCSK9 gene expression in different groups

G1 group, atherosclerosis group; G2 is normal control group. The vertical coordinate illustrates the spread of PCSK9 gene expression, with different colours signifying different groups. $p < 0.05$, the significance of the two groups passed the Wilcoxon test.

Figure 1: Difference of PCSK9 Gene Expression between the Atherosclerotic Group and the Normal Group

4.2 The expression level of PCSK9 protein in the aorta of rats in each group was detected.

Theoretically, Pcsk9-shRNA has the potential to silence the PCSK9 gene in model rats. An examination of the protein level of PCSK9 in rat aorta tissue through Western blotting demonstrated a marked increase in the expression of PCSK9 in the model group when compared to the control group (P=0.000), and the protein expression of PCSK9 was significantly reduced in the high-fat+PCSK9-shRNA group compared to the model group (P=0.000). It can be seen that the PCSK9 gene of rats was successfully knocked down. The level of PCSK9 gene expression in the aortic plaque of rats from Group A and Group B was determined, as illustrated in Figure 2.



(1) Control group; (2) Model group; (3) High-fat + shRNA-NC; (4) High-fat + Pcsk9-shRNA* P=0.000, compared with the control group; * * p= 0.000, compared with the model group.

Figure 2: Detection of PCSK9 Gene expression in Aortic Plaques of Four Groups of Rats

5. Discussion

Arthritis is a long-term inflammatory condition and the atherosclerotic plaques it brings can be incredibly detrimental to one's health. Studies have shown that late-forming plaques can cause myocardial infarction, stroke and peripheral artery disease [10]. The purpose of this study was to explore the role of PCSK9 gene in the formation and occurrence of atherosclerosis.

Data mining was employed to forecast the variance in PCSK9 gene expression between different groups. By utilizing PCSK9-shRNA interference model rats induced via a high cholesterol diet, we investigated the role of PCSK9 gene in the genesis and progression of atherosclerosis. Data mining showed that there was a significant difference in the expression of PCSK9 gene between the atherosclerotic group and the control group. This result is further confirmed by subsequent experiments. Li Qing et al. discovered that PCSK9 is linked to neuronal apoptosis, and a bioinformatics analysis revealed the presence of a Hi-1 α binding site in the PCSK9 promoter region. The data mining technique employed is analogous to ours. This unsurprising result is because data mining has become an important part of genetic analysis of the human genome, which can identify new targets for genetic diseases and validate previously discovered disease targets [11-15].

Subsequent experiments have corroborated the findings of data mining. The PCSK9 expression level in the atherosclerotic group was notably higher than that in the control group. These data further support that PCSK9 is a crucial gene involved in the development of atherosclerosis. According to Zhang Haina [16], the proprotein converting enzyme subtilysin (PCSK9) not just modulates blood lipid metabolism in an indirect manner, but also has a direct effect on AS by being involved in lipid accumulation, inflammation and apoptosis of vascular parietal cells. According to the experiment of Xu Jiabin and his team [17], PCSK9 protein interrupts the circulation of LDLR on the cell membrane, thus causing the emergence of AS. Our research team documented a case of LDLR gene mutation in which the blood lipid levels were not regulated effectively, which eventually resulted in a series of cardiovascular events [18]. This study's results are analogous to those of the other study. This is not difficult to explain. By hindering low-density lipoprotein cholesterol through LDLR transport, the plasma LDL-C is augmented, thus leading to the deposition of arterial plaque. The expression of PCSK9 in the PCSK9 knockout group was markedly reduced in comparison to the atherosclerotic group. This outcome can be attributed to the silencing of the PCSK9 gene, which has the potential to reduce the inflammatory response in AS plaques, thus preventing the progression of AS by decreasing the production of inflammatory cytokines.

6. Conclusions

In conclusion, our previous data mining found that there was a significant difference in the expression of PCSK9 gene between the atherosclerotic group and in the control group. Further investigations confirmed that the level of PCSK9 in the atherosclerosis group was notably higher than that in the control group. The expression of PCSK9 in the PCSK9 knockout group was significantly lower than in the atherosclerotic group. It is evident that PCSK9 gene plays a significant role in both apoptosis and plaque formation.

References

[1] Monahan RC, Beaart-Van D, Steup-Beekman GM, et al (2017). Neuropsychiatric symptoms in systemic lupus erythematosus: impact on quality of life. Lupus, 1252-1259.

[2] Wei T, Pan X, Silu HE, et al (2016). Clinical effect of traditional Chinese medicine in the treatment of patients with atherosclerosis and cerebral infarction. China Medicine and Pharmacy, 1(12):12-15.

[3] Pang Q, Jin H, Ke X, et al (2020). The Role of Serotonin in Concanavalin A-Induced Liver Injury in Mice. Oxid Med Cell Longev. Jan 6; 7504521.

[4] Wang Anlu, Li Qiuyi, Xu Hao, et al (2020). Relationship between intestinal flora and atherosclerosis. Chinese Journal of Arteriosclerosis28 (02):93-98.
[5] Latimer J, Batty JA, Neely RD, et al (2016). PCSK9 inhibitors in the prevention of cardiovascular disease. J Thromb Thrombolysis. Oct; 42(3):405-19.
[6] Hu D, Yang Y, Peng DQ. Increased sortilin and its independent effect on circulating proprotein convertase subtilisin/kexin type 9 (PCSK9) in statin-naive patients with coronary artery disease. Int J Cardiol. 2017 Jan 15; 227:61-65.
[7] Stoekenbroek RM, Lambert G, Cariou B, et al (2018). Inhibiting PCSK9 - biology beyond LDL control. Nat Rev Endocrinol. Dec; 15(1):52-62.
[8] Rouse WB, Andrews RJ, Booher NJ, et al (2022). Prediction and analysis of functional RNA structures within the integrative genomics viewer. NAR Genom Bioinform. Jan 14; 4 (1): lqab127.
[9] Tang Y, Tang Z, Yang J, et al (2021). MicroRNA-7-5p Inhibits Migration, Invasion and Metastasis of Intrahepatic Cholangiocarcinoma by Inhibiting MyD88. J Clin Transl Hepatol. Dec 28; 9(6):809-817.
[10] Liu F, Zhu H, Cheng X (2021). Vespa Mandarinia Recognition and Prediction Strategy Based on Geographic Location and Image Recognition. IOP Conference Series: Earth and Environmental Science, 769(3):032063.
[11] Li Qing (2018). The role and mechanism of PCSK9 in neuronal apoptosis induced by hypoxia. Nanhua University,
[12] Zhao XM, Gao JX, Nacher JC (2014). Data mining in translational bioinformatics. Biomed Res Int.; 2014:656519.
[13] Zhou H, Tian J, Sun H, et al (2022). Systematic Identification of Genomic Markers for Guiding Iron Oxide Nanoparticles in Cervical Cancer Based on Translational Bioinformatics. Int J Nanomedicine. Jun 29; 17:2823-2841.
[14] Sethi P, Theodos K (2009). Translational bioinformatics and healthcare informatics: computational and ethical challenges. Perspect Health Inf Manag. Sep 16; 6 (Fall):1h.
[15] Yan Q. (2010). Translational bioinformatics and systems biology approaches for personalized medicine. Methods Mol Biol.; 662:167-78.
[16] Zhang Haina, Wang Xiaojing, Guo Zhen, et al (2018). Research progress on the mechanism of atherosclerosis induced by PCSK9. Chinese Journal of Gerontology, 38(03):759-761.
[17] Xu Jiabin, Xie Lingpeng, Wang Libo (2018). Effects of PCSK9 on cellular biological function in the process of atherosclerosis. South China Journal of Defense Medicine, 32(06):421-424.
[18] Yang L, Xiao YY, Shao L, et al (2022). Proprotein convertase subtilisin/kexin type 9 inhibitor non responses in an adult with a history of coronary revascularization: A case report. World J Clin Cases. Jul 6; 10(19):6728-6735.