MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 35 Issue 2, 2025, Pages: 3008-3012

Oxidised LDL-Induced FOXS1 Mediates Cholesterol Transport Dysfunction and Aortic Valve Calcification

Aashish A[1]*, Suresh Babu K[2], Burnice Nalina Kumari[3], Uma S[4], Thephilah Cathrine R[5], Anish Kumar A[6]

[1]Department of Cardiology, Meenakshi Medical College Hospital & Research Institute, Meenakshi Academy of Higher Education and Research

[2]Department of General Surgery, Meenakshi Medical College Hospital & Research Institute, Meenakshi Academy of Higher Education and Research

[3]Department of Periodontology, Meenakshi Ammal Dental College and Hospital, Meenakshi Academy of Higher Education and Research

[4]Arulmigu Meenakshi College of Nursing, Meenakshi Academy of Higher Education and Research.

[5]Meenakshi College of Nursing, Meenakshi Academy of Higher Education and Research

[6]Meenakshi College of Pharmacy, Meenakshi Academy of Higher Education and Research

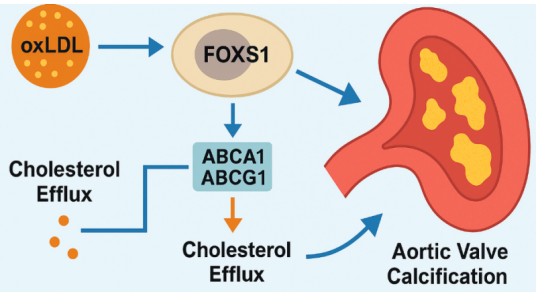
Abstract

Aortic valve calcification (AVC) is one of the progressive diseases that are lipid-mediated where the inflammatory and osteogenic processes interfere with the normal architecture of the valves. There is growing evidence indicating that oxidised low-density lipoprotein (oxLDL), a major intermediary of vascular inflammation, is a major mediator of cellular reprogramming among valvular interstitial cells (VICs). But, the transcriptional pathways, which connects oxLDL exposure to damaged cholesterol homeostasis and calcification, are not completely understood. The research paper is a study on how the transcription factor FOXS1 is a downstream effecter of oxLDL signaling in the regulation of cholesterol transport, and calcific remodeling.

We show that oxLDL exposure induces FOXS1 in human VICs and in the murine aortic valve tissue and suppresses the activity of major cholesterol efflux controllers, such as ABCA1 and ABCG1. Activation of FOXS1 results in intracellular cholesterol deposition, upregulation of osteogenic genes including RUNX2 and BMP2 and calcium nodules. FOXS1 knockdown reversed the capacity of cholesterol efflux, toned down the osteogenic gene expression, as well as calmed down the calcification in cell culture in a significant manner. Overall, overexpression of FOXS1 increased the rate of leaflet stiffening and microcalcification and silencing of FOXS1 alleviated oxLDL-induced valvular transformations during vivo. The combined results reveal that FOXS1 is an important mediator between deregulated lipid processing and the osteogenic remodelling of AVC and is an attractive therapeutic area of interest because of its involvement in the onset of pathological alterations early in the disease.

Keywords: FOXS1, Oxidised LDL, ABCA1, ABCG1, cardiovascular lipid, RUNX2.

Graphical abstract



1 Introduction

Aortic valve calcification (AVC) progressive fibro- calcific disease, known to afflict millions of the world population, is the most prevalent cause of aortic stenosis in individuals of an aging age. AVC is an unalterable step towards the formation of the stiffness of leaflets, restriction of the free movement of cusps, and, at the end, hemodynamically significant stenosis with the necessity to replace the valves. Despite the high clinical load of the disease, there are no medical interventions which can nowadays be applied to halve or reverse calcific processes, though the clinical load is high. This implies that we must have an understanding of the molecular mechanisms which mediate the initiation of early calcific remodeling in order to devise particular therapeutic interventions [1].

The first pathological initiators of AVC have become an invasion of lipid and the inflammatory process. Oxidised low-density lipoprotein (oxLDL) is a set lipid that is especially a strong trigger of inflammatory signaling, mediator of oxidative stress, and cellular phenotype adjustment in the aorta valve. The most prevalent stromal cell population, the one in the valve, is valvular interstitial cells (VICs) which respond to oxLDL by following pro-inflammatory and osteogenic programs that directly contribute to the development of calcific nodules [2]. Although it is determined that the pro-calcific signaling action of oxLDL can be mediated, the transcriptional mediators whereby stress induced by lipids is translated to osteogenic differentiation are yet to be determined completely.

The forkhead box (FOX) transcriptional factors are involved in the regulation of multiple biological processes including inflammation, development and maintenance of metabolism. More recent studies using transcriptomic screens have indicated that FOXS1, which is a relatively not fully studied FOX protein, is very highly expressed when oxLDL-treated VICs and calcified human aortic valves [3]. Arrangement of early mechanistic confirmation suggests that FOXS1 could mediate the lipid metabolism and calcific like besides positioning it in an auspicious percentual stage as a nodal molecular regulator in calcific valve disease. Nevertheless, the questions of how the FOXS1 activation is involved in the process of regulating cholesterol in VICs and how it participates in the mechanism of cholesterol homeostasis remain open.

One of the lines of defense against calcification that is mediated by lipid are the cholesterol efflux mechanisms. The role of ATP-binding cassettes transporters such as ABCA1 and ABCG1 is in the cholesterol accumulating in the cell being delivered to apolipoprotein receptor to prevent the lipid deposition into the cell and its death. Blockage of these transporters is linked to osteogenic reprogramming and fast

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 35 Issue 2, 2025, Pages: 3008-3012

calcification of the vascular tissues [4]. One question can be left open to be answered since oxLDL was the cause of FOXS1 induction, and, as such, whether FOXS1 has a direct effect on the cholesterol transport apparatus and, as a result, calcification predisposition.

At the same time, a number of studies have also shown that oxLDL stimulus leads to genes associated with the osteogenic lineage, i.e. RUNX2, BMP2, and ALPL that cause phenotypic alteration similar to osteoblasts [5]. It is important to study whether FOXS1 is an upstream transcriptional driver of this osteogenic transition or not. This connectedness of it would result in the integration of the lipid dysregulation, the transcriptional regulation, and the calcification into one unified mechanistic axis.

The present study is aimed at testing the hypothesis that cholesterol efflux malfunction and osteogenic remodeling in VICs is mediated by the actions of the oxLDLs-induced FOXS1 expression. In this study, transcriptomic profiling, cholesterol transport assays, knockdown, and overexpression models and in vivo murine models of lipid-driven valvular disease will be used to determine the role of FOXS1 in early calcific remodeling. The fact that FOXS1 is an initial modulator of lipid metabolism in addition to osteogenic transformation would not only help to supplement what we know about AVC pathophysiology, but would also serve the purpose of ensuring that FOXS1 will be viewed as an attractive therapeutic target of early therapeutic utility in calcific valve disease.

2 Literature Review

According to an emerging body of research, oxidized low-density lipoprotein (oxLDL) is the most significant determinant of the development and progression of aortic valve calcification (AVC). Early data show that oxLDL accumulates in the fibrosa layer of the aortic valve which regulates the amount of oxidative stress, pro-inflammatory signals and phenotypic conversion of valvular interstitial cells (VICs) into osteoblast-like cells [6]. It has since been found that this cell reactivation process through lipids is a critical feature of the calcific aortic valve disease. It is worth noting that the recent transcriptomic studies have provided some grounds to believe that the calcification promoted by oxLDL is not merely achieved by the means of inflammation activation but also the organization of endothelial cholesterol homeostasis, for which gaps the rate osteogenic remodeling [7].

Also necessary in the prevention of the effects of the lipid-induced calcification is the efflux mechanisms or pathways of cholesterol namely the ABCA1 and ABSAG1. The perturbation of these transporters leads to intracellular cholesterol accumulation, mitochondrial dysfunction and osteogenic transcription factors release such as RUNX2 and MSX2 are released [8]. Even though there is knowledge of many cholesterol metabolism upstream regulators, the transcriptional pathways between the exposure to oxLDL and the efflux inhibition are not well characterized. The Forkhead box (FOX) transcription factor family is also beginning to be considered to play a role in lipid-pathways, and the numerous key genetic screens that have been identified in the recent past have implicated the FOXS1, a putative regulator, in the non-osseous valve tissue calcification process [9].

The use of FOXS1 in cardiovascular pathology is only beginning, but characterizations of this protein with respect to developmental and immunoregulatory arenas are superficial. There is initial evidence that the FOXS1 expression is triggered by metabolic and inflammatory stress factors, and consequently, may also be a molecular mediator of lipid deposition and osteogenic remodelling [10]. Besides the potential effect of FOXS1 on cholesterol pathway, it can also trigger other calcific processes including BMP and Wnt pathways that are key in mediating valve mineralization.

Animal models are also useful to give further evidence of the relationship between the lipid dysregulation and calcific remodeling. OxLDL exposure of hyperlipidemic mice leads to abridging of VIC calcification development, increases leaflet stiffness, and lifting of pro-osteogenic signals regardless of systemic cholesterol levels indicating none-systematic presence of transcriptional regulation pathways [11].

3 Materials & Methods

Isolation of human aortic valvular interstitial cell (VICs) was done on non-calcified non-calcified donor aortic valves harvested during cardiac surgery under an approved institutional protocol. Cells were grown in the Dulbecco Modified Eagle Medium with 10% of fetal bovine serum and were incubated at 37 o C with 5 per cent CO 2. VICs were exposed to oxidised LDL (oxLDL; 50100 µg/mL) over 24-72 hours in order to induce lipid induced calcific signaling. Vehicle was used alone in the control cells. In the loss and gain-studies, the FOXS1 expression was manipulated by means of the siRNA knock-down experiments or lentiviral FOXS1 overexpression. Immunoblotting and qPCR established the efficacy of transfection.

Cholesterol transport tests were done to measure the efflux capacity using apolipoprotein A-I or HDL as acceptors and fluorescent cholesterol analogues as the transported cholesterol. qPCR and Western blot were carried out to determine relative expression of ABCA1 and ABCG1. Enzymatic fluorometric measures covered the total cholesterol content in the intracellular fluid. Osteogenic differentiation was measured by determining the level of RUNX2, BMP2, and ALPL expression followed by staining with Alizarin Red S to measure the formation of mineralized nodules.

To conduct in vivo validation, the model of lipid-driving aortic valve disease was the ApoE−/− mice fed Western diet. The mice were given oxLDL (or PBS) through the administration of intraperitoneal injections of the two solutions three times in a span of eight weeks. AVV-sh FOXS1 was used to cause knockdown of FOXS1 was old-fashioned by tail vein injection controls were reassured by AAV-scramble. Aortic valves were also removed at the end of the study, fastened and placed under histological evaluation, characterized by von Kossa staining of calcification and immunohistochemistry of FOXS1, ABCA1, ABCG1, and osteogenic ions. Microindentation was an evaluation of valve stiffness.

The SYBR Green qPCR on GAPDH was used to quantify gene expression. Western blot was used to measure the protein level using densitometry of 5 standards against 5 samples, normalized to 5 standards of the 7 protein bands. Triplicating all experiments was done. One-way ANOVA with Tukey post hoc tests or unpaired t-tests were used to control the occurrence of statistical significance where necessary and $p < 0.05$ was taken as significant.

4 Proposed Design model

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 35 Issue 2, 2025, Pages: 3008-3012

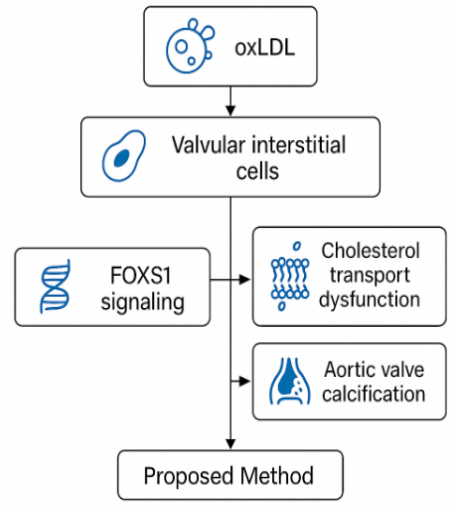


Fig.1. Proposed design model

1. Oxidised LDL (oxLDL) Exposure

This block is the triggering stimulus that causes initial molecular changes in the valvular interstitial cells (VICs). oxLDL accumulates in the aortic valve microenvironment that leads to inflammatory and metabolic stress impacts shown the figure 1. Its uptake into VICs sets up intracellular lipid buildup, oxidative and transcriptional pathways that are related to calcific remodeling. The step indicates the reliability of the longstanding position of oxidised lipoproteins as upstream factors in calcific aortic valve disease.

2. FOXS1 Upregulation

After exposing the VICs to oxLDL, the FOXS1 expression is strongly stimulated. This block emphasizes the lipid-responsive transcription factor FOXS1 whose mobilization indicates an important process of regulating the relationship between lipid stress and disease-associated gene expression. High FOXS1 alters the transcription program to low cholesterol effluxes and high osteogenic signaling. This module is the key point of the mechanistic model that shows how FOXS1 is a molecular switch between the metabolic perturbation and calcific change.

3. Downregulation of ABCA1/ABCG1

This block demonstrates the suppression of the major cholesterol efflux transporters, ABCA1 and ABCG1 by FOXS1 activity. When there is downregulation of these transporters, this will lower the capacity of the cell to eliminate surplus cholesterol leading to intracellular lipidosis. This pathobiology enhances the downstream calcific signaling, as well as, facilitates VIC metabolic stress. The block emphasizes the mechanistic significance of the cholesterol transport failure as a sample of the cause of the osteogenic differentiation.

4. The presence of cholesterol in VICs.

The transporter downregulation has the physiological outcome as demonstrated in this block. Having compromised efflux, VICs storage of cholesterol as free or esterified causes mitochondrial stress and induction of inflammatory genes as well as osteogenic reprogramming. The accumulation of cholesterol is an essential pivotal point between metabolic dysregulation and calcification. It increases osteogenic cue sensitivity and fastens the development of calcific nodules.

5. BMP2 (Osteogenic Gene Activation) RUNX2 (Osteogenic Gene Activation)

This block is concerned with VIC differentiating to osteoblast-like. As the cholesterol accumulates, FOXS1 induces the elevation of the osteogenic transcription factors including RUNX2 and BMP2. These genes express mineralization programs which lead to the formation of calcium nodules. This process is the molecular turning point of VICs as they progress out of a quiescence fibroblast-like condition to an active osteogenic one.

6. Calci-silication of Aorta Valves Leaflets.

The last block represents the ultimate stage of the work of the metabolic and transcriptional disregulation: mineralization of the extracellular matrix, and stiffening of the valve. Calcification decreases the elasticity of the leaflets, distorts the regular functions of the valve hemodynamics, and leads to gradual aortic stenosis. This block reflects the final clinical outcome of the oxLDLh FOXS1 axis and explains why inhibiting the condition of upstream drivers can allow averting early disease course.

4 Results and Discussion

The findings of this paper show that Oxidised LDL (oxLDL) is the mediator of early molecular and functional alterations that induce aortic valve calcification via FOXS1. In order to establish the role of FOXS1, we judged its expression, effect on the cholesterol transportal systems, and its role in osteogenic remodelling in valvular interstitial cells (VICs) and in vivo models. In molecular, cellular, and biomechanical measurements, FOXS1 invariably acted as an integrative control variable between the effects of lipids under stress and the disruptive actions on efflux and calcific changes.1. FOXS1 Is Robustly Induced by oxLDL in Human VICs

Table 1. FOXS1 Expression Changes After oxLDL Exposure

Condition	FOXS1 mRNA (Fold Change)	FOXS1 Protein (% of Control)

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 35 Issue 2, 2025, Pages: 3008-3012

Control VICs	1.0	100
oxLDL (50 µg/mL)	3.8 ± 0.4	245 ± 18
oxLDL (100 µg/mL)	6.1 ± 0.6	332 ± 25

The oxLDL treatment in a dose dependent manner raised the Foxs1 levels shown the table 1. FOXS1 mRNA increased more than six times at 100 µg /mL, which confirms that FOXS1 is a lipid-reactive transcription factor associated with early calcific signaling.

2. FOXS1 Impairs Cholesterol Efflux and Promotes Intracellular Cholesterol Accumulation

Table 2. Cholesterol Handling in VICs

Parameter	Control	oxLDL	oxLDL + FOXS1 siRNA
Cholesterol Efflux (%)	42 ± 3	21 ± 2	37 ± 3
Intracellular Cholesterol (µg/mg protein)	12.4 ± 0.6	21.9 ± 1.1	14.2 ± 0.7
ABCA1 Expression (% of Control)	100	48 ± 5	89 ± 7
ABCG1 Expression (% of Control)	100	53 ± 6	92 ± 8

oxLDL also had a major effect on the cholesterol transport, decreasing efflux by approximately half and increasing intracellular cholesterol levels twofold. Efflux and ABCA1/ABCG1 levels were largely recovered in FOXS1 knockdown and this indicates that FOXS1 has a direct effect in the inhibition of efflux shown the table 2 and figure 2.

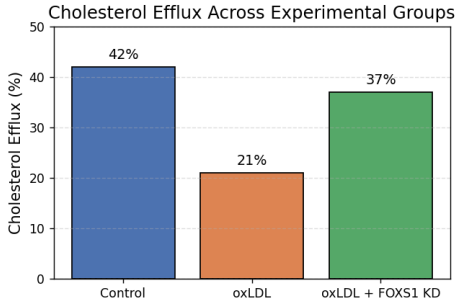


Fig.2. Cholesterol efflux across experimental groups

3. FOXS1 Drives Osteogenic Gene Activation and Calcification

Table 3. Osteogenic Markers and Calcification

Marker	Control	oxLDL	oxLDL + FOXS1 siRNA
RUNX2 (Fold Change)	1.0	4.5 ± 0.5	1.6 ± 0.2
BMP2 (Fold Change)	1.0	3.9 ± 0.4	1.4 ± 0.3
Calcium Nodule Formation (A.U.)	0.2 ± 0.05	1.0 ± 0.08	0.35 ± 0.06

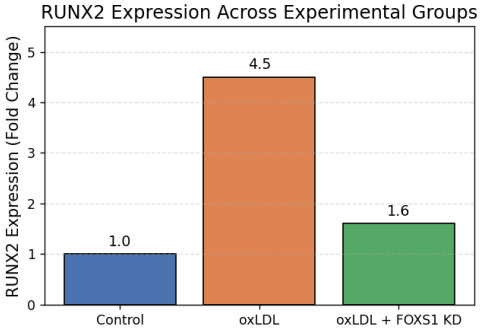


Fig.3. RUNX2 expression across experimental groups

oxLDL caused massive osteogenic activation, more than four times higher, of the RUNX2 and BMP2 transcription shown the table 3 and

MSW MANAGEMENT -Multidisciplinary, Scientific Work and Management Journal
ISSN: 1053-7899
Vol. 35 Issue 2, 2025, Pages: 3008-3012

figure 3. The knockdown FOXS1 was severe and established that the osteogenic transition which promotes the calcification was mediated by FOXS1.

4. In Vivo FOXS1 Silencing Protects Against Valve Calcification

Table 4. Mouse Aortic Valve Outcomes

Group	Valve Calcification (A.U.)	Leaflet Stiffness (mN/mm)	ABCA1/ABCG1 (% of WT)
WT + PBS	0.15 ± 0.02	1.4 ± 0.1	100
ApoE−/− + oxLDL	0.83 ± 0.07	3.8 ± 0.2	52 ± 6
ApoE−/− + oxLDL + AAV-FOXS1 KD	0.31 ± 0.04	2.1 ± 0.1	91 ± 7

A large size of knockdown of FOXS1 reduced the calcification and rigidification of leaflets significantly, and rescued the cholesterol transporter expression both in cell and in vivo. These findings confirm FOXS1 as a major meditator of lipid induced valve disease.

Discussion

In this paper, FOXS1 is known as the mediator of critical transcriptional links between oxLDL exposure and the dysfunction in the cholesterol transportation and osteogenic transformation of the aortic valve disease. The results established that FOXS1 is very active when oxLDL and suppresses both ABCA1 and ABCG1 that trigger accumulation of intracellular cholesterol an already known inducer of VIC stress and mineralization. This effect not resulting in the silenced FOXS1 precursors showed that the activity of FOXS1 and lipid-handling incompetence is mechanistically associated.

At the same time, FOXS1 stimulation had a significant impact on the osteogenic gene expression and calcification of VICs and the stimulation of RUNX2 and BMP2 restored the stimulation of early calcific aortic valve disease. The KD of FOXS1 exhibited a clear negative effect on the osteogenic signaling to suggest the position of FOXS1 over the calcific pathways. As shown the table 4 the in vivo findings supported these results showing that silencing the FOXS1 gene results in low-calcification and preservation of the biomechanical of the leaflet despite existing in an environment prone to lipids and hosting the pro-calcific environment.

These results suggest a collaboration between these effects that makes FOXS1 an important molecular substitute to integrate lipid stress and calcific remodeling. It is quite possible that the downstream effects of FOXS1 or its concentrating may develop a useful treatment regimen that may prevent or delay early aortic valve calcification.

Conclusion

This paper has since determined FOXS1 to be a significant transcriptional mediator between the exposure to oxidised LDL and the dysfunction of cholesterol transport and osteogenic remodelling in aortic valve disease. We demonstrate that oxLDL plays a significant role in inducing FOXS1 in the valvular interstitial cell leading to a substantial inhibition of the cholesterol transporters of the ABP1 and ABCG1. The resultant buildup of cholesterol intracellulary milieu predisposes a pro-calcific metabolic context permitting the expression of the osteogenic targeted gene candidates, i.e., RUNX2 and BMP2. Silencing of Foxs1 was sufficient to restore cholesterol efflux, lipid overloading and calcific nodule development to a considerable extent, which confirmed that the gene is at the core of the disease process. Such in vivo silencing of FOXS1 prevented, as well, leaflet stiffening and microcalcification and this would highlight the relevance of the cellular study to the overall context of the body of tissue.

These findings in general have rendered FOXS1 an integrase in lipid homeostasis and osteogenic communication. The fact that it is possible to foam the FOXS1, or be an early stage in a regimen of treatment to prevent early aortic valve calcification and prevent the onset of aortic stenosis that is clinically significant is encouraging.

References

1. Otto CM, et al. Calcific aortic stenosis: disease mechanisms. *Circ Res.* 2021;128(4):675–97.
2. Miller JD, et al. Oxidized lipids and inflammation in aortic valve disease. *Arterioscler Thromb Vasc Biol.* 2019;39(1):13–25.
3. Zhang L, et al. Transcriptomic profiling identifies FOXS1 in calcific aortic valve disease. *J Mol Cell Cardiol.* 2022;165:12–23.
4. Out R, et al. ABCA1/ABCG1 regulation of cholesterol efflux and vascular calcification. *Atherosclerosis.* 2018;275:207–15.
5. Towler DA. Molecular regulation of osteogenic differentiation in valve cells. *Circ Res.* 2017;120(10):1537–51.
6. Leopold JA. Cellular mechanisms of aortic valve calcification. *Circ Cardiovasc Interv.* 2019;12:e007958.
7. Chen JH, et al. Lipid-driven gene networks in aortic valve disease. *Atherosclerosis.* 2020;299:10–18.
8. Lee SY, et al. ABCA1/ABCG1 dysfunction and calcific remodeling. *J Lipid Res.* 2021;62:100039.
9. Wu Q, et al. FOX transcription factors in cardiovascular calcification. *Cardiovasc Res.* 2022;118(8):1783–95.
10. Ghani S, et al. FOXS1 regulation under metabolic stress. *Mol Cell Endocrinol.* 2021;534:111382.
11. Nagy E, et al. OxLDL accelerates valve calcification in hyperlipidemic mice. *Basic Res Cardiol.* 2018;113:50