Vascular Health and Risk Management

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REVIEW

FSTL-1 as a Novel Cardiokine of Cardiac Angiogenesis: A Systematic Review

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Background: Follistatin-like 1 (FSTL1) is recently becoming a novel cardiokine essential in cardiac angiogenesis. This cardiokine has shown a potential to promote angiogenesis and improve cardiac function, particularly in myocardial injury and ischemia. Despite the increasing relevance, there is no information on the mechanisms of FSTL1 in cardiac angiogenesis.

Objective: This systematic review aimed to consolidate recent results on the role of FSTL1 in molecular pathways of cardiac angiogenesis.

Methods: A comprehensive search was conducted using various databases, including PubMed, Scopus, SpringerLink, and ScienceDirect. Inclusion criteria were primary studies that investigated the role of FSTL1 in promoting cardiac angiogenesis with in vivo models. The risk of bias was assessed using SYRCLE risk of bias tool, and data were synthesized to evaluate the impact of FSTL1 on cardiac angiogenesis.

Results: A total of 5 animal studies were included during the analysis. The results showed the role of FSTL1 as a novel cardiokine in inducing cardiac angiogenesis as assessed by protein examination and histologic analysis. In pathological conditions, the effects of ischemia on the heart increased the expression of FSTL1 as a form of protection for the heart through angiogenesis and as a marker of the disease severity. Furthermore, the molecular mechanisms of FSTL-induced angiogenesis had different signaling pathways, including activation of AMPK, TGFβ-Smad2/3, Akt/mTOR, Erk1/2, and DIP2A-PI3K. Studies showed increased capillary density and improved blood flow in cardiac tissues where FSTL1 was upregulated, suggesting a possible important role in improving cardiac function.

Conclusion: FSTL1 showed a promising avenue for therapeutic development. Moreover, future studies should explore its role in cardiac angiogenesis in healthy populations.

Keywords: angiogenesis, cardiovascular, FSTL1 protein, follistatin-related proteins, gene expression

Introduction

Cardiovascular disease is the leading cause of death in the world, causing an estimated 17.9 million deaths each year. More than 80% of deaths from cardiovascular disease are caused by heart attacks and strokes, where one-third occur at a young age or less than 70 years.[1] The increase in the number of cases due to cardiovascular disease has led to decreased quality of life and high healthcare costs.[2] Currently, the increasing trend of cardiovascular disease is followed by unhealthy lifestyle behaviors such as smoking, lack of physical activity, disruption of rest time, and poor food consumption patterns including high sugar and cholesterol.[3,4] This is accompanied by an increase in hypertension, obesity, hypercholesterolemia, and diabetes mellitus, which are major causes of cardiovascular disease and stroke.[5,6] Generally, it is characterized by narrowing or blockage of blood vessels leading to impaired blood supply, and most commonly caused by atherosclerosis.[7] Therefore, cardiovascular disease treatment options are currently focused on re-establishing blood flow through the affected blood vessels.[7,8]

Received: 16 December 2024
Accepted: 13 April 2025
Published: 27 May 2025

Given the poor angiogenic properties of the heart in patients with vascular disorders, there is a need for novel therapeutic approaches to promote cardiac regeneration by targeting genes involved in angiogenesis.[9] Thus, gene-based therapies that utilize pro-angiogenic factors may be a promising therapeutic strategy. Moreover, many current therapies are designed without targeting and considering the specific molecular pathways involved in cardiac regeneration, making them less effective. Failure to utilize gene targets is also due to the lack of knowledge about the molecular mechanisms of disease. This is the reason why cardiovascular diseases continue to grow due to the use of wrong gene targets and inconsistent clinical trials.[10] Based on these clinical demands, angiogenesis plays an essential role in resupplying blood flow to impaired ischemic tissue.[11]

Therapeutic angiogenesis offers a potential method to improve ischemic tissue function by stimulating blood vessel growth and improving tissue perfusion, thereby supporting regeneration and recovery.[12] One of the efforts that can encourage the process of angiogenesis is to maximize the potential of local cardiac genes that can modulate angiogenesis. Thus, the angiogenesis process can be optimized, both in pathological and physiological conditions. This effort provides long-term sustainable benefits, as one of the therapeutic efforts by targeting therapy at specific gene targets, and can be a solution in reducing the risk of side effects due to long-term use of drugs.[13]

The process of angiogenesis can occur under normal (physiological) or stressful (pathological) conditions. One of the pathways that plays a role in cardiac angiogenesis is HIF-1 (Hypoxia-Inducible Factor 1), which has been widely studied to have a synergistic correlation with angiogenic factors, including VEGF (Vascular Endothelial Growth Factor).[14] Hypoxic conditions can stimulate the release of HIF-1, so many studies state that the process of cardiac angiogenesis depends on oxygen or hypoxia, or also called the HIF-1-dependent pathway.[15] However, recent studies have revealed that there are other pathways independent of HIF in regulating angiogenesis through VEGF expression, namely PGC-1α (Peroxisome proliferator activated receptor-gamma coactivator 1 alpha) and FSTL1 (Follistatin-like 1) which are not yet widely known.[16–18] The independent HIF-1 pathway occurs without direct hypoxia involvement. However, it is triggered through mechanical and metabolic factors by involving other molecules that stimulate angiogenesis without the need for hypoxia.[19]

Several molecules can potentially induce protection in cardiovascular system.[20] Recently, FSTL1 is a cardiokine that has gained significant attention and is widely recognized due to its effects on the heart.[21–23] FSTL1, also known as TSC-36 (Follistatin-related polypeptide), is a secreted glycoprotein that belongs to the follistatin family of proteins.[24] It is secreted at various sites, with the most secretion found in the heart, muscle, and adipose tissue, which is called adipo-cardio-miokine.[25–28] Specific to the heart, FSTL1 is produced from various cells, such as cardiomyocytes, fibroblasts, and endothelial cells, but most secretions are found in cardiomyocytes.[29,30] This cardiokine also plays an important role in improving heart function by reducing the area of infarction/fibrosis,[17,31] preventing cardiac pathological remodeling,[19,32] promoting fibroblast proliferation to prevent heart structure rupture,[33] and as an anti-inflammatory agent.[34]

Another important role of FSTL1 in cardiac protection is as a pro-angiogenesis gene.[31] In the process of angiogenesis, FSTL1 in the heart is more likely to work indirectly, although there is some evidence to suggest that FSTL1 also plays a direct role. Directly, FSTL1 can interact with various growth factors and receptors involved in angiogenesis. FSTL1 acts as a modulator that affects the expression of genes related to the formation of new blood vessels.[17,31] Indirectly, FSTL1 works through regulating other molecular pathways that support angiogenesis by reducing inflammation and inhibiting fibrosis.[19,32,34] FSTL1 interacts with the DIP2A receptor and activates the Smad2/3 signaling pathway, which is important for angiogenesis. This pathway is independent of TGFβR1, thus highlighting the unique mechanism by which FSTL1 promotes endothelial cell proliferation and blood vessel formation.[18] FSTL1 is also found in the blood-stream contributing to angiogenesis and cardioprotection. These systemic effects underscore the importance of FSTL1 as a circulating cardiokine. In turn, FSTL-1 will induce other angiogenic factors such as VEGF, FGF, and NDNF, to promote cardiac remodeling and angiogenesis.[35]

In particular, the increased expression of FSTL1 was found to be more significant in pathological conditions such as myocardial infarction, as it is crucial in tissue repair and angiogenesis.[36] Consequently, it is considered a pro-survival cardiokine acting as a diagnostic marker and treatment simultaneously.[33,37] Overexpression of FSTL1 in rats has shown positive cardiac angiogenesis. It can even activate vascular endothelial growth factor (VEGF), the primary pro-angiogenic gene of the heart.[18] In comparison, heart-specific FSTL1 knock-out rats were shown to develop cardiac

hypertrophy and ventricular dysfunction in response to transverse aortic constriction.[32] The recent literature review only explores the effects of FSTL1 on inflammation,[38] cardiac structural remodeling,[39] atherosclerosis,[40] proliferation, and fibrosis.[41] The ability of FSTL1 to promote angiogenesis, makes it a promising candidate for gene therapy aimed at improving blood flow and cardiac function, both in physiological and pathological conditions.[24,38,42]

Although the potential effects on cardiac angiogenesis have been explored, there is limited information on the biomolecular mechanisms of FSTL1. In another study, there were contradictory results that FSTL-1 expression can play a role in the activation of cardiac fibroblasts, potentially leading to fibrosis of the myocardium.[36] These conditions can disrupt cardiac angiogenesis through changes in tissue structure, decreased bioavailability of angiogenic factors, and impaired endothelial cell proliferation. This difference in results suggests that the role of FSTL-1 in angiogenesis may be influenced by various pathological condition-specific factors as well as the mechanisms of FSTL-1 induction in the heart that need to be known biomolecularly. Therefore, this systematic review aimed to explore the biomolecular mechanism of FSTL1 as a potential therapeutic in cardiac angiogenesis.

Material and Methods

Design

This study used a systematic review design using Preferred Reporting Item for Systematic Reviews and Meta-Analysis (PRISMA) framework.[43]

Search Strategy

A comprehensive literature search was conducted using PubMed, Scopus, SpringerLink, and Science Direct databases published from October 2011 to September 2021. The search strategy used keywords and Medical Subject Headings (MeSH) to identify two general concepts, namely FSTL1 and angiogenesis. Some keywords were correlated with Boolean operators "OR" and "AND" for keywords in the same and different concepts, respectively. The various keywords included fstl1 OR "fstl1 protein" OR "fstl gene product" OR "follistatin like protein 1" OR "follistatin-like protein 1" AND angiogenesis OR "angiogenesis protein" OR "angiogenesis effects" OR neovascularization. The search process was limited to articles published in English. This literature review aimed to identify all studies related to the role of FSTL1 in cardiac angiogenesis. The range of data collection was conducted from September to October 2024. More details can be seen in the extraction Table S1 in the Supplementary Files.

Study Selection

All search results were downloaded and entered into Rayyan software to check for duplication. The three reviewers (PK, SS & NF) independently screened all search results, starting with selecting titles and abstracts based on inclusion and exclusion criteria, and conducting a full-text assessment. When there was a difference of opinion between the three reviewers, the problem would be resolved by including a third reviewer (NS) to assess the study.

Inclusion and Exclusion Criteria

The inclusion criteria were determined based on the PEO (population, exposure, and outcome) formulation. Specifically, the population was animal models, exposure to reporting the results of molecular mechanisms including FSTL1, and the outcome included showing a picture of cardiac angiogenesis. Studies were excluded when there was no focus on cardiac angiogenesis, non-English, studies in the review, meta-analysis, or editorial design, and needed more data for analysis.

Data Extraction and Analysis

The three reviewers (PK, NF, SS) independently extracted data from the study, with a high agreement of ≥95% between the three reviewers. Any disagreement was resolved by consensus with an other reviewer (NS). Subsequently, one reviewer (PK) extracted data from all included studies. The extracted data were presented as a table, including the author and year of publication, design and sample characteristics, and main results related to the role of FSTL1 in angiogenesis process.

Quality Assessment

Quality assessment was performed with SYRCLE (Systematic Review Centre for Laboratory Animal Experimentation) for studies using animal models to ensure there was no bias. A total of two reviewers (PK and SS) independently assessed the risk of bias. SYRCLE risk of bias tool assessment included selection (sequence generation, baseline characteristics, and allocation concealment), performance (randomized and masked housing), detection (randomized and masked outcome), subtraction (incomplete outcome data), reporting bias (selective outcome reporting), and other sources of bias.[44] The assignment of low, high, or unclear risk of bias assessment referred to the following options: "low" showed a low-risk of bias, "high" indicated a high-risk of bias, and "unclear" suggested insufficient or unreported details to assess the risk of bias properly. In case of disagreement, resolution was performed through joint discussion with the third reviewers (NS).

Results

Study Selection

A total of 639 studies were identified from PubMed (n = 31). Scopus (n = 28), Springer Link (n = 287), and Science Direct (n = 293). Based on analysis, 48 studies were duplicates, and only 591 articles were screened. Furthermore, 569 studies were excluded by title and abstract, with 22 studies remaining eligible for full-text review. From 22 studies analyzed, 15 were excluded due to the failure to meet the inclusion criteria such as review articles, wrong population and outcomes, and no full-text article. Finally, 5 in vivo studies were included in this systematic review as shown in PRISMA flowchart (Figure 1).

Characteristics of Included Studies

These studies have various variations in design, population, and analysis method, with results focusing on the role of FSTL1 as a novel cardiokine in cardiac angiogenesis. The results showed that all studies used rats as the sample (n = 5; 100%) and were published between 2011 and 2021. It was reported that the samples were given certain inductions, including FSTL1 knockout rats (n = 1; 20%), diabetes mellitus (n = 1, 20%), and myocardial infarction (n = 3; 60%). The sample analysis method in all studies used Western blot (n = 4; 80%), and the remaining applied ELISA test (n = 1; 20). The results showed the expression of FSTL1 at protein level, describing actual and accurate biological effects of gene expression on biological activity, as shown in Table S2.

FSTL1 Gene Expression on Cardiac Angiogenesis

A total of 5 studies described the effect of FSTL1 on cardiac angiogenesis in animal models. All results showed that FSTL1 played an important role in cardiac angiogenesis (n = 5; 100%). The studies also supported the results with histological examinations that showed an increase in the growth of new blood vessels along with an increase in FSTL1 expression.[17,18,31,32,35] Furthermore, FSTL1 expression increased in pathological conditions as a form of cardiac biological defense against compromised blood vessels, along with exercise induction of various types, duration, and intensity. This showed that FSTL1 acted as a biomarker as well as a treatment in preventing and treating vascular disease in the heart. Although FSTL1 shows a positive impact on angiogenesis, there is still limited information compared to other markers. Through signaling and receptor regulation, this cardiokine can promote other angiogenesis markers such as VEGF.[18,35] The results show that FSTL1 is an important cardiokine to be considered a novel marker of angiogenesis in the heart.

On histological examination, all studies revealed structural changes in the heart of rats that have increased FSTL1 expression compared to the control and knock-out groups. The study of Shimano et al[32] showed that in the TAC (transverse aortic contraction) group that had increased FSTL1 protein, the heart appeared structurally normal and increased LVEDD (Left Ventricle end diastolic diameter) compared to the control. In contrast, FSTL1-KO (FSTL-1 knock out) rats showed myocyte hypertrophy, fibrosis and reduced myocardial capillary density. AMPK phosphorylation was found to be increased in the TAC group, but reduced in the FSTL1-KO group. This indicates that AMPK activation plays a role in pressure overload conditions in the heart. FSTL-KO rats also showed a decrease in eNOS phosphorylation,

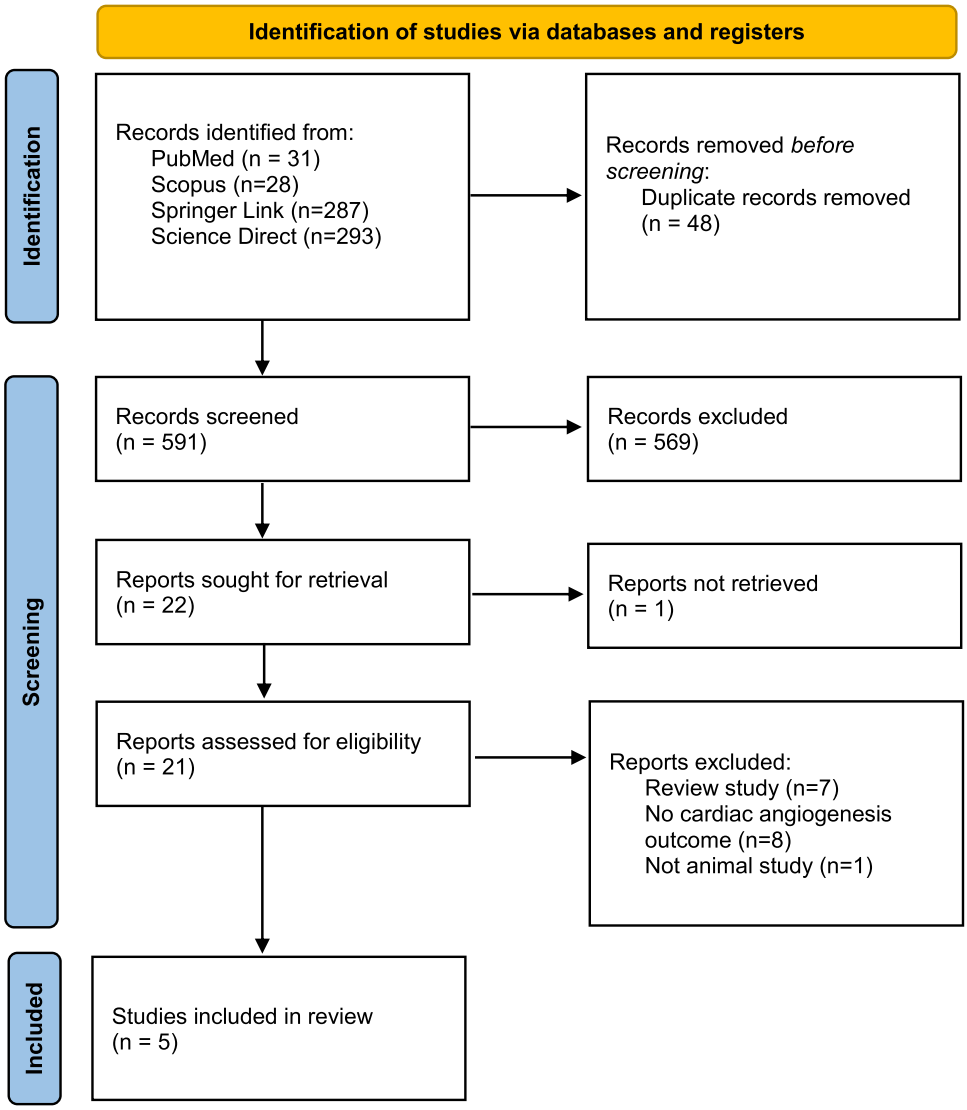

Figure 1 PRISMA flowchart of the study selection process. Adapted from Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372.[43]

but no change in cardiac SMAD-2 phosphorylation. Similar results were found in the study of Arabzadeh et al[35] using diabetes mellitus rats found that the insulin group that experienced an increase in FSTL1 found vascular improvement. Better results were found in the exercise and insulin groups, where there was an increase in the number and diameter of new blood vessels. But on the contrary, in the control group the number and diameter of blood vessels decreased and there was instability in the tissue.

In the other three studies, studies were conducted on myocardial infarction samples and induced with exercise. The study of Xi et al[31] showed increased expression of FSTL1 forming new small blood vessel-like structures. FSTL1 also induced CD31 proliferation strengthening cardiac capillaries. To look at angiogenesis, this study examined endothelial cell proliferation by co-staining PCNA+ cell proliferation marker, and vWF+ endothelial cell marker. Compared to the control group, MI induced more PCNA+/vWF+ cells, which increased in number and distribution due to exercise. Exogenous FSTL1 administration was found to promote the growth of more new blood vessels at the infarct site and increase Akt, Erk1/2 and AMPK signaling pathways. These results suggest that FSTL1 exerts direct cardioprotective actions by promoting angiogenesis, increasing FSTL1 protein content and activating TGFβ-Smad2/3 signaling in the post-MI heart, all of which alleviate cardiac dysfunction. Similar results were shown by the studies of Xi et al[17] and Xi et al,[18] which revealed that the exercise group with myocardial infarction had a high increase in FSTL1 expression, with histological examination results showing the number of micro-vessels increased and

surrounded each other to form the primary vascular structure. Resistance training effectively inhibited myocardial fibrosis and collagen infiltration of the myocardium, and pathological remodeling was significantly inhibited.

FSTL1 Signaling Pathways in the Process of Cardiac Angiogenesis

All the studies stated that the regulation of FSTL1 in supporting the process of cardiac angiogenesis was influenced by various signaling pathways, including AMPK,[31,32] TGFβ-Smad2/3,[17,18,31] Akt/mTOR,[31,35] Erk1/2,[17,31] and DIP2A-PI3K.[17,18] Shimano et al explored FSTL1 deficiency in rats and found that AMPK activation was weakened. In FSTL1 group, AMPK activity increased causing a corresponding rise in the phosphorylation of eNOS.[32] High AMPK activity was also reported by Xi et al, showing that FSTL1 could function as FSTL through TGFβ-Smad2/3 signaling pathway to induce angiogenesis.[31]

A similar study found increased TGFβ1 protein expression along with increased Smad2/3 levels downstream.[17] Xi et al stated that the exogenous FSTL1 administration increased 54.14% TGFβ and 76.70% Smad2/3 protein expression at week 5.[31] Similar results were found in the Akt, Erk1/2, and AMPK signaling pathways. This suggested that increased FSTL1 expression was influenced by TGFβ, Akt, Erk1/2, and AMPK signaling pathways in the process of cardiac angiogenesis.

In the process of angiogenesis, FSTL1 expression included protein kinase Akt activated by the PI3K pathway, inducing mTOR, which would directly activate and regulate eNOS to produce NO. The production of NO plays an important role in protecting endothelial function, including regulating vasoconstriction, vasodilation, and angiogenesis in cardiac blood vessels.[18,32,35] The study also found that DIP2A was considered a potent receptor of FSTL1[17,18] which bind to DIP2A to increase endothelial cell proliferation. This process focused on promoting the increase of VEGF for angiogenesis to appear in the heart.

Risk of Bias

Each study was assessed for quality using SYRCLE tool. Based on the results, 5 studies were of good overall quality, as shown in Figures 2 and 3.

Discussion

This is the first systematic review of FSTL1 effects on cardiac angiogenesis. The analysis was conducted to determine the biomolecular mechanism of FSTL1 regulation as a novel cardiokine in inducing angiogenesis in the heart. The literature

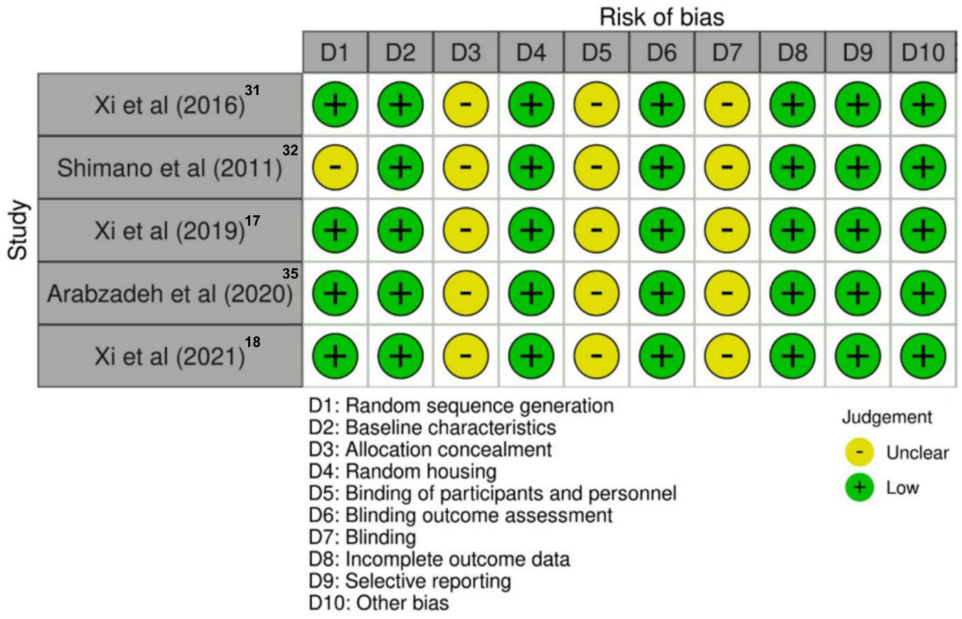

Figure 2 Traffic light plot's risk of bias.

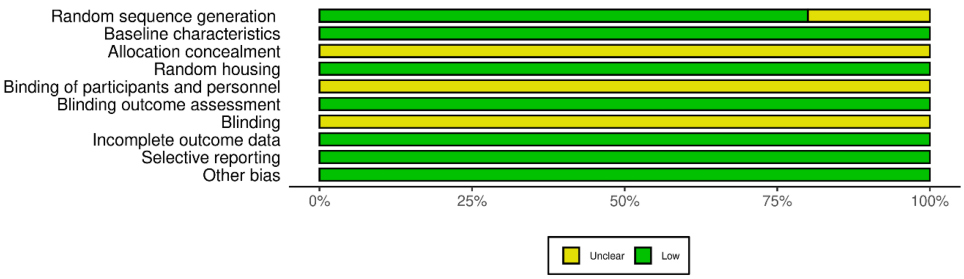

Figure 3 Summary risk of bias.

search identified five studies that met the inclusion criteria to be further analyzed and comprehensively summarized. The results showed that FSTL1 positively promoted cardiac angiogenesis in pathological conditions described in animal model with myocardial infarction and diabetes. Further analysis showed that the role of FSTL1 in angiogenesis did not occur only but included various other proteins through signaling pathways. This finding is very interesting, because FSTL1 directly promotes angiogenesis and proliferation of cardiomyocytes under hypoxic conditions or mechanical stress, while other angiogenic genes such as VEGF and HIF-1α through hypoxia-induced pathways.[29,32,41] VEGF plays a central role in the process of angiogenesis by increasing endothelial cell proliferation and vascular permeability by activating other pathways such as NFκB and HIF-1α. FSTL1 may be an upstream pathway for the induction of VEGF and other growth factors, and FSTL1's unique mechanism and multifaceted role in promoting angiogenesis and cardiomyocyte proliferation along with its anti-inflammatory properties, differentiate it from other growth factors in the context of angiogenesis.

FSTL1 is a cardiokine secreted in other organs, such as skeletal muscle and adipose tissue.[25,30] Recent studies showed that FSTL1 secreted in muscle could reach the heart through serum.[17,18] So it is not only the original FSTL1 from the heart that can promote cardiac angiogenesis but FSTL1 produced by skeletal muscle can reach the heart through the vascular, causing an angiogenesis response. Especially in the heart, previous results showed that FSTL1 expression was increased in conditions of ischemia and cardiac hypertrophy, associated with high inflammatory response and oxidative stress.[19,22] Other conditions that increased FSTL1 secretion were heart failure, myocardial infarction, aortic constriction, including during exercise.[17,25,26,32,36,45,46] A study on myocardial infarction heart with a patch containing recombinant FSTL1 showed significant improvement in heart function by increasing angiogenesis and preserving cardiomyocytes.[23] This was supported by other studies on increasing overexpression through exogenous FSTL1 injection and exercise, which showed an angiogenesis response, reduced fibrosis area, and improved cardiac recovery after myocardial infarction.[18,25] Rats with FSTL1 overexpression resisted cardiac dysfunction after transferential aortic constriction.[32] In this systematic review, there were no sample groups with healthy characteristics or without cardiovascular disease. Therefore, the effect of FSTL1 on cardiac angiogenesis as a form of preventive action should be further investigated.

The results showed that DIP2A was found to be an important receptor of FSTL1 in endothelial cells or cardiac smooth muscle cells.[17,18] When FSTL1 is secreted, there is an interaction with DIP2A as its receptor along with TGFβ.[47] Loss of DIP2A (knockout) leads to decreased phosphorylation of Akt, which plays a role in cell viability, differentiation, and migration.[47] The autocrine and paracrine activities of FSTL1 on cells depend on DIP2A as its receptor. In the autocrine mechanism, FSTL1 binding to DIP2A on the surface of the heart plays a role in cell proliferation for tissue regeneration and reduces the inflammatory response. FSTL1 produced by cardiomyocytes/cardiac fibroblast cells in paracrine binds to DIP2A in endothelial cells. This phenomenon could cause an increase in protein concentration in surrounding cells leading to angiogenesis and protection against cardiac apoptosis.[22,23,48–50] PI3K inclusion is also important in producing second messengers that play a role in Akt activation and other transduction pathways, namely phosphatidylinositol 3,4,5-triphosphate (PIP3) and phosphatase and tensin homolog (PTEN). Furthermore, PI3K activation participates in vascular formation and permeability when VEGF binds to its receptor in endothelial cells.[51]

The role of FSTL1 in protecting the heart is shown through ERK phosphorylation, which has been proven to prevent pathological vascular remodeling.[25,52] ERK is a signal transduction protein that transmits mitogenic signals and is often

located in the cytoplasm. As one of the classical MAPK signaling pathways, the Ras-Raf-MEK-ERK follows the tertiary enzymatic cascade reaction.[53,54] VEGF activation, directly and indirectly, induces angiogenesis through the Ras-Raf-MEK-ERK signaling pathway. In previous inhibitory studies, VEGF attenuates the Ras/Raf-MEK/ERK and eNOS/NO signaling pathways to reduce angiogenic response.[55] In addition, FSTL1 binding to DIP2A, through PI3K/Akt and ERK1/2 signaling pathways also increases the transcription of FGF-2 and Angiopoietin-1 genes. This plays an important role in endothelial cell migration and proliferation, supports the stabilization of new blood vessels, and enhances the interaction of endothelial cells with pericitocytes and extracellular matrix thereby aiding in the maturation and stabilization of blood vessels during angiogenesis.[56,57]

In conditions of ischemia/hypoxia due to exercise or vascular obstruction, the cell environment becomes acidic because of increased ATP demand, causing anaerobic glycolysis that produces lactic acid and ketone bodies.[58] The role of ROS (Reactive Oxygen Species) also increases due to an imbalance in production with elimination.[58] This condition will activate PI3K-Akt pathway to maintain intracellular homeostasis by inducing angiogenesis through NO and endothelial nitric oxide synthase (eNOS). Therefore, mTOR also activates to regulate the release of eNOS and NO that will induce angiogenesis response in the heart.[31,32,35,59] FSTL1 and DIP2A binding cause an increased expression of AMPK, which is a regulator of eNOS/NO.[31,60] AMPK is also recognized as a key regulator of glucose and lipid metabolism in various organs, including the heart, and enhances mitochondrial biogenesis.[61] The activation of PGC-1α by AMPK is important in enhancing mitochondrial biogenesis, energy metabolism, and cardiac angiogenesis.[62]

From the various interactions, Figure 4 summarizes how the relationship between FSTL1, angiogenesis, and its signaling pathways in the heart. Under injury or ischemic conditions, the body will secrete FSTL1 as a form of defense against the heart. FSTL1 binds to DIP2A receptors on endothelial cells, which will mediate cardiac angiogenesis by promoting the survival, migration, and differentiation of endothelial cells into tissue structures.[47] The binding of FSTL1 to DIP2A directly activates the Smad2/3 and TGFβ signaling pathways, which also suggests a potential feedback

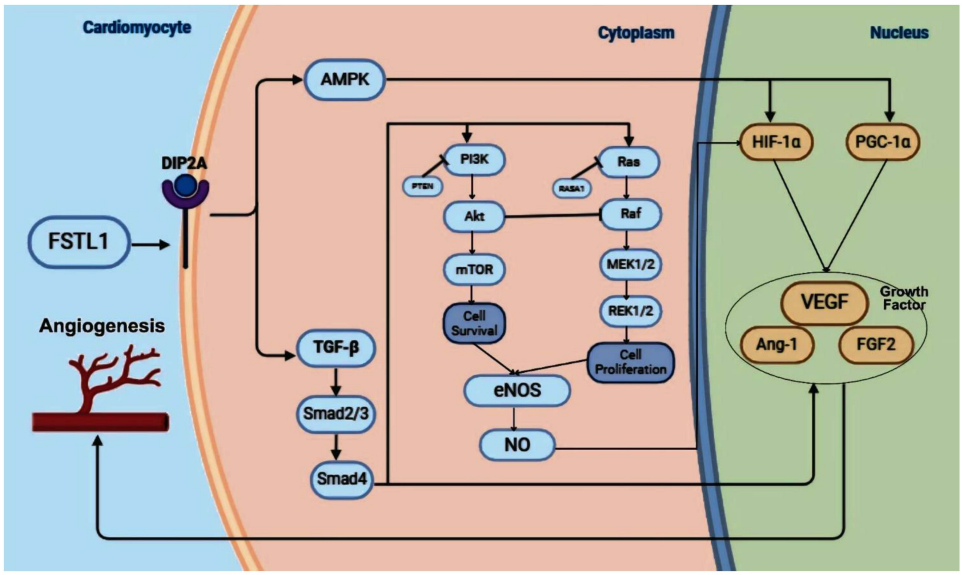

Figure 4 Proposed Mechanism of FSTL1 on cardiac angiogenesis.

Notes: Plausible mechanisms of FSTL1 on cardiac angiogenesis. FSTL1 will bind to DIP2A as its main receptor. It then induces 2 pathways directly, namely TGFβ and AMPK. TGFβ will phosphorylate Smad2/3 and activate Smad4, which further induces angiogenic factors, one of which is VEGF. VEGF binds to VEGFR-2 as a receptor, which activates the PI3K/Akt/mTOR and Ras/Raf/MEK1/2/REK1/2 pathways that induce endothelial cell proliferation and migration. The pathway phosphorylates eNOS to NO, which will induce HIF-1a as a hypoxia factor. HIF-1a will induce various growth factors, such as VEGF, FGF2, and Ang-1, resulting in an angiogenesis response. While in the AMPK pathway, it will induce HIF-1a and PGC-1a to directly activate various angiogenesis growth factors. AMPK activation is regulated in injury conditions such as ischemia and infarction as well as due to exercise.

Abbrevations: FSTL1, Follistatin-Like Protein 1; DIP2A, Disco interacting protein 2 homolog A; AMPK, AMP-activated protein kinase; PGC-1α, Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1 Alpha; Ang-1, Angiopoietin 1; FGF2, Fibroblast Growth Factor-2; VEGF, Vascular Endothelial Growth Factor; HIF-1α, Hypoxia-Induced Factor 1 Alpha; TGFβ, Transforming Growth Factor Beta; PI3K, Phosphatidylinositol-3 kinase; PTEN; Akt, Protein kinase B; mTOR, mammalian target of rapamycin; Ras, Rat sarcoma virus; Raf, Rapidly accelerated fibrosarcoma; MEK1/2, Mitogen activated protein kinase kinase; REK1/2, Extracellular Signal-Regulated Kinase 1/2; eNOS, endothelial Nitric Oxide Synthase; NO, Nitric Oxide.

loop.[24,63] FSTL1 can induce angiogenesis through overlapping pathways with TGF-β signaling, while TGF-β can increase FSTL1 expression to regulate vascular stability and repair.[18,24] TGFβ inhibition can reduce Smad2/3 phosphorylation and VEGF expression, which inhibits angiogenesis.[18] Smad 2/3 will bind to Smad4, which then moves to the nucleus to regulate the expression of pro-angiogenic genes, one of which is VEGF.

The relationship between VEGF and the RAS/PI3K/Akt pathway can be bidirectional. VEGF when binds to VEGFR2 in endothelial cells will activate the PI3K/Akt and Ras/Raf/MEK/ERK pathways that play a role in increasing endothelial cell proliferation and migration, by activating eNOS to NO.[18,47] On the other hand, binding of VEGF and VEGFR2 in endothelial cells can induce the Ras pathway, which will activate PI3K through PIP3. Ras then activates Raf, which further phosphorylates and activates MEK. MEK, in turn, phosphorylates and activates ERK to carry out cellular responses. MEK can regulate PTEN, a phosphatase that controls Akt signaling. Inhibition of the ERK pathway can lead to increased Akt activation due to reduced PTEN activity.[64] Both pathways can also be activated simultaneously by certain stimuli such as mTOR.[65] This then activates Akt, which will phosphorylate eNOS to NO. NO has functions in vasodilation and vascular stabilization so that new vascular homeostasis can be maintained.[66,67] The release of eNOS and NO also stimulates HIF-1α as a hypoxia inducing factor, which further induces vascular growth factors such as VEGF, FGF2, and angiopoietin. Then, separately under these conditions, FSTL1 also regulates the phosphorylation of AMPK as a metabolic sensor. AMPK increases the release of eNOS and NO, which then activates HIF-1α and mitochondrial biogenesis (PGC-1α) which further induces VEGF.[32,68]

This study has proven that FSTL1 can regulate cardiac angiogenesis through various molecular mechanism pathways. Clinical studies using human populations have also found high levels of FSTL1 in heart failure, acute coronary syndrome, and healthy subjects with inflammation and oxidative stress.[69–72] However, the effect of FSTL1 in the myocardium and its function on cardiac angiogenesis remains unexplored. A study of 231 myocardial infarction patients at 2 weeks and 6 months after infarction showed an increase in FSTL1 in the myocardial infarction patient group indicating damage. However, this study did not assess its cardioprotective effect after infarction.[73] Another study of 32 patients with heart failure found that the group with increased FSTL1 levels had lower pulmonary artery pressure and right atrial pressure. Kaplan–Meier survival analysis revealed that the low myocardial FSTL1 mRNA expression group had a low survival rate compared to the high one ($p = 0.013$). From the findings of this study, it is also known that the expression of FSTL1 in the myocardium is low when compared to transcardiac FSTL1, there is a decrease of about 50%, suggesting that there are other mechanisms that can produce FSTL1 other than the heart. This is explained because although FSTL1 expression is accelerated under pathological conditions, FSTL1 consumption is also increased.[74] Although FSTL1 has protective effects, its overexpression or dysregulation can lead to adverse outcomes, such as excessive fibrosis and hypertrophy, so it is important to maintain balanced FSTL1 expression as a protective strategy for the heart.[32,41,75]

Although FSTL1 plays a role in cardiac angiogenesis, it is insufficient in long-term cardioprotection, particularly in recovery from myocardial infarction. Therefore, various intervention modalities are carried out to support the long-term increase in FSTL1 expression, such as exercise.[31] A total of 4 among 5 studies including samples of induced cardiovascular disorders that were given exercise interventions showed an effect of exercise on cardiac angiogenesis. This was because exercise had a muscle ischemia-like effect through hypoxia, contributing to increased circulating levels of FSTL1.[31] Exercise is a powerful stimulator of muscle growth, metabolism, and endocrine function.[76] The recommended exercise modalities to maintain cardiac angiogenesis still need to be determined. Recent review studies showed that moderate-intensity exercise had an impact on atherosclerosis repair.[40] Furthermore, this type of exercise along with resistance training was recommended for providing cardiac remodeling effects.[39] A meta-analysis showed that in human populations, individuals who performed aerobic exercise with resistance training had a lower mortality risk, than only one type.[77] In line with WHO (World Health Organization) recommendations, combining all types of exercise (aerobic and resistance) into one session showed high effectiveness.[78] However, when compared to each type of exercise, it was found that aerobic exercise had a stronger effect on vascular function and angiogenesis than resistance training, both in clinical trials and in vivo studies.[79,80] Nevertheless, regardless of the type of exercise, it can improve cardiovascular function by inducing an angiogenic response in the myocardium.[79] Especially resistance training requires further research

due to the limited evidence available.[39] The significant effect of FSTL1 in healthy groups is still unknown, creating opportunities for future studies regarding the impact of exercise on cardiac angiogenesis physiologically.

Conclusion

The results showed that FSTL1 expression in the heart increases in pathological conditions such as ischemia. This occurs because FSTL1 acts as a form of physiological protection of the heart against blood vessel disorders. However, limited levels of FSTL1 from the heart make it functionally not optimal so that FSTL1 levels in the heart need to be stabilized as needed. One thing that can be done is exercise. Exercise helps induce FSTL1 produced by the muscles to enter the heart through the blood vessels. Unfortunately, all studies used pathological animal populations so that physiological exercise-induced FSTL1 function was not found. Future studies should explore how FSTL1 affects angiogenesis under physiological conditions to provide new insights. In addition, FSTL1 overexpression also causes adverse effects on the heart. However, none of the studies found an optimal level of FSTL1 in the heart. Therefore, future research can investigate the correlation of FSTL1 levels in cardiomyocytes and cardiac myokines with the angiogenesis process through observational studies. Future studies can also consider Mendelian randomization studies to reveal the relationship between FSTL1 gene polymorphisms and the risk of developing cardiovascular disease, thus increasing the potential for future clinical trials.

Acknowledgments

The authors are grateful to Universitas Padjadjaran Internal Grant [1607/UN6.3.1/PT.00/2024] for NS and the PMDSU Scholarship for funding the publication of this systematic review.

Disclosure

The authors declare no conflict of interest in this work.

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