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REVIEW ARTICLE

Follistatin-like 1 in Cardiovascular Disease and Inflammation

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Abstract: Follistatin-like 1 (FSTL1), a secreted glycoprotein, has been shown to participate in regulating developmental processes and to be involved in states of disease and injury. Spatiotemporal regulation and posttranslational modifications contribute to its specific functions and make it an intriguing candidate to study disease mechanisms and potentially develop new therapies. With cardiovascular diseases as the primary cause of death worldwide, clarification of mechanisms underlying cardiac regeneration and revascularization remains essential. Recent findings on FSTL1 in both acute coronary syndrome and heart failure emphasize its potential as a target for cardiac regenerative therapy. With this review, we aim to shed light on the role of FSTL1 specifically in cardiovascular disease and inflammation.

Keywords: Follistatin-Like 1, FSTL1, cardiac regeneration, inflammation, cardiovascular diseases, glycoprotein, cardiomyocytes.

1. INTRODUCTION

Follistatin-like 1 (FSTL1), also known as TSC-36 and follistatin-related protein (FRP), was first discovered in an osteoblastic cell line as a gene induced by transforming growth factor (TGF) β1 expression [1]. This 308 amino acid extracellular glycoprotein is a member of the SPARC (secreted protein acidic rich in cysteine) family of proteins [2, 3] and has a molecular mass ranging from 40 to 55 kDa [4, 5]. Analysis in mice indicated it to be most abundantly expressed in the lungs [6], the heart [7], skeletal muscle [8], smooth muscle [9], and vascular endothelium [9]. Multiple effects of FSTL1 have been reported, including a regulatory role in embryogenesis [10], inhibition of proliferation in cancer cells [11], and modulation of inflammatory responses [6]. Mechanistically, these various roles of FSTL1 are mediated through different pathways targeting BMP for developmental processes, AKT/AMPK in the context of cardiac disease and CD14/TLR4 for immunological processes [12].

1.1. Cardiokines as Potential Therapeutic Targets for Heart Failure

Heart failure represents a significant health burden within the spectrum of cardiovascular diseases affecting approximately 26 million people worldwide [13]. The treatment options are limited which leads to a poor prognosis with a five-year survival rate of 51.5% [14]. Over the last years, cardiovascular research on heart failure has focused on methods to repair the heart. The use of stem cells in the context of cardiac regeneration has been studied extensively. However, the results of (pre-)clinical trials using stem cell therapy are inconsistent [15]. The main obstacles that are encountered are the complex molecular mechanisms underlying cardiac repair, but also the delivery method [15], low cellular retention [16] and functional integration [17]. Furthermore, the focus on repair of the injured myocardium is accompanied by undervalued importance of the restoration of the microvasculature of the heart and the functional integration of the injected cells within the host myocardium and the extracellular matrix [18]. The use of extracellular vesicles has also received interest as the vesicles can mediate paracrine effects and multiple reparative functions, including stimulating angiogenesis and cardiomyocyte proliferation [19]. There is increasing evidence suggesting that endogenous factors secreted from cardiac tissue, referred to as cardiokines, play an essential role in regulating cellular mechanisms in the heart [20]. They can function through autocrine, paracrine, and endocrine signaling and are involved in responses to injury, cardiac remodeling, and inter-cellular and inter-organ communication [21-25].

1.2. FSTL1 in Cardiac Disease

A protective effect of FSTL1 has been reported in various cardiac diseases (Fig. **1**) and development [26]. In tran-

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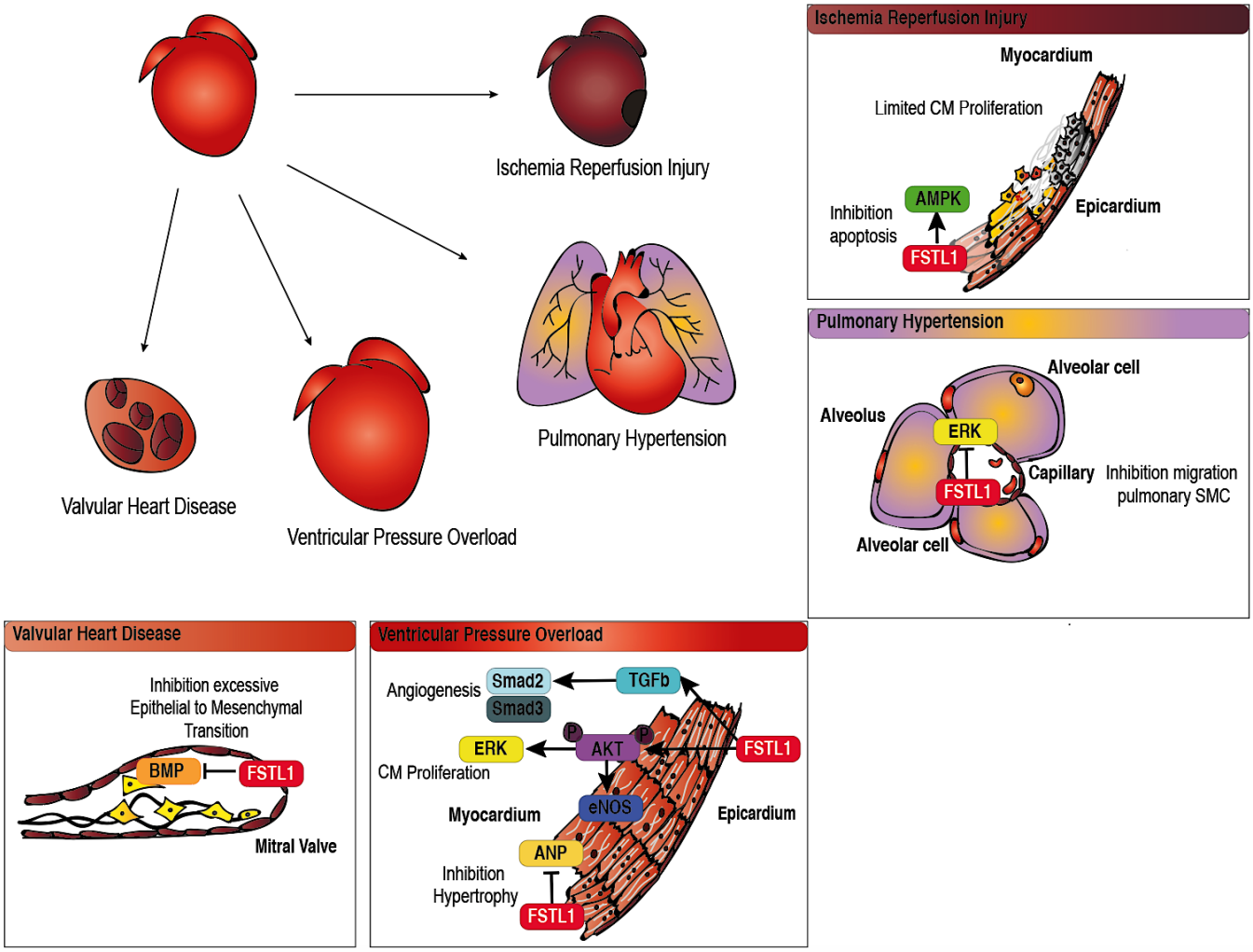

Fig. (1). The role of FSTL1 in cardiovascular diseases. In the injured heart, FSTL1 can influence multiple processes through the interplay with distinct molecular pathways in multiple cardiac cell types. In the healthy and the hypertrophied heart, the epicardial cells secrete FSTL1 inhibiting hypertrophy, stimulating cardiomyocyte proliferation and inducing angiogenesis. Epicardial FSTL1 expression is abolished during ischemia reperfusion injury. Myocardial FSTL1 can inhibit cardiomyocyte apoptosis. In valvular heart disease FSTL1 regulates epithelial to mesenchymal transition. In pulmonary hypertension, FSTL1 inhibits the migration of pulmonary smooth muscle cells (SMC).

saortic constricted hearts, FSTL1 reduces cardiomyocyte apoptosis via phosphorylated AMPK signaling [20]. Furthermore, the loss of FSTL1 in transaortic constricted hearts is associated with more severe myocardial hypertrophy and loss of ventricular function, emphasizing a role for FSTL1 in maintaining cardiac function [27]. Similarly, in infarcted hearts FSTL1 protects from apoptosis and induces angiogenesis via phosphorylated AKT signaling [7, 28, 29]. For the stimulation of angiogenesis, phosphorylated AKT signaling is dependent on the interaction between FSTL1 and transmembrane protein DIP2A, which functions as a receptor for FSTL1. A 22% increase in the secretion of FSTL1 by skeletal muscle fibers during exercise could also be the underlying mechanism of cardioprotection acquired through exercise [8]. The capacity to modulate cardiovascular diseases via both cardiac secreted FSTL1 and skeletal muscle secreted FSTL1 indicates the potential role of FSTL1 in myocardial repair after injury.

1.3. FSTL1 may Enhance Cardiac Function Following I/R Injury

1.3.1. Cardioprotection

FSTL1 has been described as an active modulator of cellular responses in various cardiac conditions and may have the capacity to generate both cardioprotective and regenerative responses. Previous studies showed that FSTL1 acts as a cardioprotective factor after ischemia/reperfusion (I/R) injury. FSTL1 overexpression attenuated apoptosis in neonatal rat cardiomyocytes after exposure to hypoxia and reoxygenation. Additionally, systemic administration of recombinant FSTL1 after myocardial infarction in mice significantly reduced the rate of cardiomyocyte apoptosis and the size of the infarct [30]. Similarly, anti-apoptotic effects of FSTL1 have been shown *in vitro* and in murine and porcine I/R models [30]. Upregulation of endogenous FSTL1, as a response to exercise, attenuated cardiac fibrosis, while intrape-

ritoneal administration of FSTL1 was associated with increased systolic function in a rat model of myocardial infarction [31]. There is increasing evidence suggesting that these cardioprotective properties may be mediated by activation of AMP-activated protein kinase signaling [30, 32]. Furthermore, a recent study shows that FSTL1 expression is suppressed by miRNA-9-5p, a non-coding RNA which is upregulated after ischemic injury [33]. Antagomir mediated inhibition of miRNA-9-5p in a murine acute MI model attenuated fibrosis and inflammation and preserved cardiac function.

1.3.2. Proliferative Responses

Recently, FSTL1 was found to act as a regenerative factor by promoting proliferation of mouse embryonic stem cell-derived cardiomyocytes *in vitro* [34] raising hopes to unleash the limited endogenous regenerative capacity of the adult human heart [35] beyond the reported 0.8% of cardiomyocytes dividing annually to maintain homeostasis [36]. This baseline proliferation rate can be increased to some extent, for instance by signaling from cellular damage induced by the increased concentration of reactive oxygen species (ROS) in response to ischemia/reperfusion injury [37], via the Nrg1/ErbB2 pathway [38] and via the Hippo-YAP pathway [39], but stimulation of cardiomyocyte proliferation to allow for substantial restoration of the myocardium was considered illusive until recently. However, analysis in a mouse model of MI revealed a switch in FSTL1 expression from epicardial to myocardial cells going along with a switch in posttranslational modification from the hypoglycosylated to the hyperglycosylated isoform [34]. Since this isoform has been shown *in vitro* to be cardioprotective (anti-apoptotic) but not regenerative (proliferative), reconstitution of hypoglycosylated FSTL1 expression was mediated by surgical application of a FSTL1-loaded collagen-based patch to the epicardium immediately after the induction of myocardial infarction. Restoring epicardial FSTL1 expression significantly reduced scarring, stimulated cardiomyocyte proliferation, and enhanced cardiac function following ischemic injury in murine and porcine models [34]. FSTL1 induced increase of GDF-15 expression and activation of TGFβ-Smad2/3 signaling seems to be a potential pathway for the induction of cardiomyocyte proliferation [31, 40].

These results indicate that the decrease of epicardial FSTL1 is an adverse reaction to ischemic stress and that reconstitution comprises a promising method to preserve cardiac function and attenuate cardiac remodeling. However, a recent study found no evidence of epicardial FSTL1 expression in a *Fstl1*-eGFP reporter mouse, but could confirm FSTL1 dependent activation of cardiac fibroblasts preventing post-myocardial-infarction ventricular wall rupture [41].

1.3.3. Stimulatory Effects on Revascularization

Besides inhibiting apoptosis and stimulating proliferation of cardiomyocytes, FSTL1 may also attenuate ischemia/reperfusion injury by inducing revascularization. Recent studies have demonstrated that FSTL1 promotes angiogenesis during ischemic stress through activation of Akt-eNOS-dependent signaling [42], and TGFβ-Smad2/3 after myocardial infarction [31]. Epicardial injection of FSTL1 following myocardial infarction resulted in increased vascularization of the myocardium, particularly in the border zone of the infarcted area [34]. Furthermore, there is evidence suggesting that FSTL1 enhances endothelial cell function and survival [42] and plays a role in vascular remodeling following arterial injury by preventing neointimal hyperplasia [43].

1.4. FSTL1 as Biomarker: Elevated Expression in Acute Coronary Syndrome and Heart Failure

FSTL1 is strongly expressed under cardiac hypoxic conditions and has been proposed to be a potential diagnostic biomarker in acute coronary syndrome (ACS) and heart failure.

Patients with ACS showed serum concentrations of FSTL1 increased by 88% compared to healthy controls [40]. Upregulation of FSTL1 in ACS patients was independently correlated with an increased incidence of diabetes mellitus, increased N-terminal pro-B-type natriuretic peptide (NT-proBNP) and c-reactive protein (CRP) levels. Serum FSTL1 levels above the median were associated with reduced survival one year after ACS compared to those below the median [$P < 0.019$]. In a cohort of 106 ACS patients, patients were 3.7 times more likely to die from a cardiovascular cause when their serum FSTL1 concentration was within the top quartile compared to patients with levels in the three lower quartiles [$P < 0.001$]. The prognostic value of FSTL1 in ACS showed similar discriminatory potential as traditional markers CRP and NT-proBNP [40]. Furthermore, GDF-15, which is induced by FSTL1, has strong prognostic value for patients with non-ST-elevation ACS [44].

FSTL1 concentrations are increased in patients with ischemic and dilating cardiomyopathy with a left ventricular ejection fraction of less than 40%. In these systolic heart failure patients, serum FSTL1 levels were elevated by 56% compared to matched controls and associated with increased left ventricular mass, left ventricular posterior wall thickness, and increased brain natriuretic peptide levels [45]. Moreover, significantly elevated serum FSTL1 levels have been found in patients with end-stage heart failure with a left ventricular assist device implantation [9] and in patients with heart failure with preserved ejection fraction (HFpEF) [46]. A recent study showed that the upregulation of FSTL1 expression in heart failure could be accurately detected by using gold nanoparticles. According to the authors, the advantages of this technique are the low costs and rapid execution compared to current methods to diagnose heart failure, e.g. NT-proBNP and echocardiography [47].

The efficacy of FSTL1 as a biomarker, especially in relation to established clinical biomarkers and the association with cardiovascular events and mortality, shows promise, but still requires additional research. The increased circulation of FSTL1 in heart failure might suggest further increasing circulating FSTL1 would not improve cardiac function. Contrary to this hypothesis, a recent study showed acute or chronic FSTL1 infusion normalized cardiac metabolism and improved diastolic and contractile function [48].

1.5. Attenuation of Cardiac Hypertrophy

FSTL1 has been identified as an active modulator of cardiac ventricular hypertrophy. In a mouse model of ventricular pressure overload induced by transverse aortic constriction (TAC), cardiomyocyte-specific knock-out of FSTL1 resulted in excessive hypertrophy and deterioration of systolic ventricular function [27]. In contrast, transgenic mice overexpressing FSTL1, both conditionally and constitutively, were refractory to developing cardiac hypertrophy in this model. Moreover, systemic administration of FSTL1 decreased the hypertrophic response, enhanced systolic function, and attenuated left ventricular dimensions in both wild-type and FSTL1-knockout (FSTL1-KO) mice. TAC resulted in a 2.2 fold increase of FSTL1 serum levels and this increase was attenuated in the myocyte-specific FSTL1-KO mice, suggesting that cardiomyocytes are the main source of FSTL1 in mice subjected to TAC [27].

The ability of FSTL1 to prevent hypertrophic responses has also been demonstrated *in vitro* using adult rat ventricular cardiomyocytes [46]. Supplementation of FSTL1 to cell culture attenuated cardiomyocyte hypertrophy induced *in vitro* by d-aldosterone and reduced expression of atrial natriuretic peptide (ANP), a marker of cardiomyocyte hypertrophy. This was confirmed *in vivo* showing that increased systemic levels of FSTL1 from transgenic overexpression were associated with reduced cardiac hypertrophy and improved diastolic function in a mouse model of heart failure with preserved ejection fraction (HFpEF) [46].

1.6. Protection from Pulmonary Hypertension

Recent evidence suggests that FSTL1 also plays a role in attenuating pulmonary hypertension [49]. During pulmonary hypertension, high blood pressure in the arteries of the lungs increases the workload of the heart and can lead to right ventricular heart failure. In patients with pulmonary hypertension due to chronic obstructive pulmonary disease FSTL1 serum levels are elevated. When comparing heterozygous FSTL1+/– knock-out mice with wild type FSTL1+/+ mice, in a state of hypoxia induced pulmonary hypertension, the heterozygous mice with decreased FSTL1 expression showed higher right systolic ventricular pressures and increased right ventricular hypertrophy. Systemic administration of FSTL1 improved right-sided systolic pressures [49]. Additionally, endothelium-derived FSTL1 appears to regulate the development and remodeling of the pulmonary vasculature and loss of this type of FSTL1 may result in a reduction of right ventricular function [50].

1.7. FSTL1 and Valvular Heart Disease

Limited evidence is available regarding the potential association of FSTL1 and valvular heart disease. Recently, it has been described that endocardial and endothelial deletion of FSTL1 resulted in severe deformation of the mitral valve in a mouse model [51]. FSTL1 is normally expressed in both mitral valve leaflets and inhibits bone morphogenetic protein (BMP) signaling to prevent excessive proliferation of valve cells. Echocardiography of a conditional FSTL1 knockout mouse showed mitral regurgitation and progressive left ventricular dysfunction, eventually leading to cardiovascular death [51].

FSTL1 is associated with cardiomyocyte and endothelial cell function, and its pleiotropic function renders its potential to modulate specific processes, including cardiomyocyte survival, cardiomyocyte proliferation and angiogenesis. Additionally, modulation of FSTL1 expression can improve or worsen cardiac function in multiple cardiac diseases. Therefore, FSTL1 has the potential to serve as a therapeutic target to treat I/R injury, pulmonary hypertension, cardiomyocyte hypertrophy, and valvular heart disease.

1.8. Inflammation in Cardiac Diseases

An increasing body of evidence supports the essential role of inflammation in both the development and the progression of cardiovascular diseases [52]. Acute myocardial infarction is often caused by the formation of a thrombus on atherosclerotic plaques in coronary arteries, a process involving the activation of platelets, the accumulation of immune cells and systemic and local inflammatory events [53]. In the ischemic myocardium, injured myocytes release their intracellular content resulting in a well-orchestrated signaling cascade of neutrophil and monocyte infiltration. Reperfusion of the infarcted area leads to I/R injury mediated by the release of ROS, inducing leukocyte chemokine upregulation. The infiltration of immune cells mediates the secretion of pro-inflammatory cytokines tumour necrosis factor (TNF), IL-1β, and IL-6. The knockdown of TNF ameliorated myocardial I/R injury indicating the role of inflammation in the pathogenesis of I/R injury [54]. But the inflammatory response in the heart after ischemia is not only detrimental for cardiac function: extensive evidence suggests the involvement of inflammatory mechanisms in post-infarction cardiac repair through the clearance of dead cells [55] and also mediating regeneration by macrophages in the neonatal heart [56]. Furthermore, in a mouse model of cardiac pressure overload due to TAC, hypertrophy and myocardial inflammation preceding fibrosis was observed [57]. Additionally, Inflammation plays a role in the pathogenesis of pulmonary hypertension where endothelial dysfunction is accompanied by the upregulation of pro-inflammatory cytokines IL-1, MCP-1 and IL-6 [58].

1.9. FSTL1 in Inflammation

The identification of FSTL1 as an autoantigen in the synovium of patients with rheumatoid arthritis (RA) [4] lead to increased interest to study FSTL1 in inflammatory diseases. Follow-up studies identified both pro-inflammatory and anti-inflammatory effects of FSTL1 (Fig. **2**).

In multiple inflammatory diseases (e.g. RA [59, 60].

Sjögrens syndrome, ulcerative colitis, systemic lupus erythematosus, systemic sclerosis, and dermatomyositis / polymyositis, asthma [61]) FSTL1 levels were found to be increased and associated with disease progression. Expression of FSTL1 was found to be specifically increased in mesenchymal lineage cells and not in the hematopoietic lineage in patients with RA. The plasma levels of FSTL1 were increased in acute Kawasaki disease compared to healthy

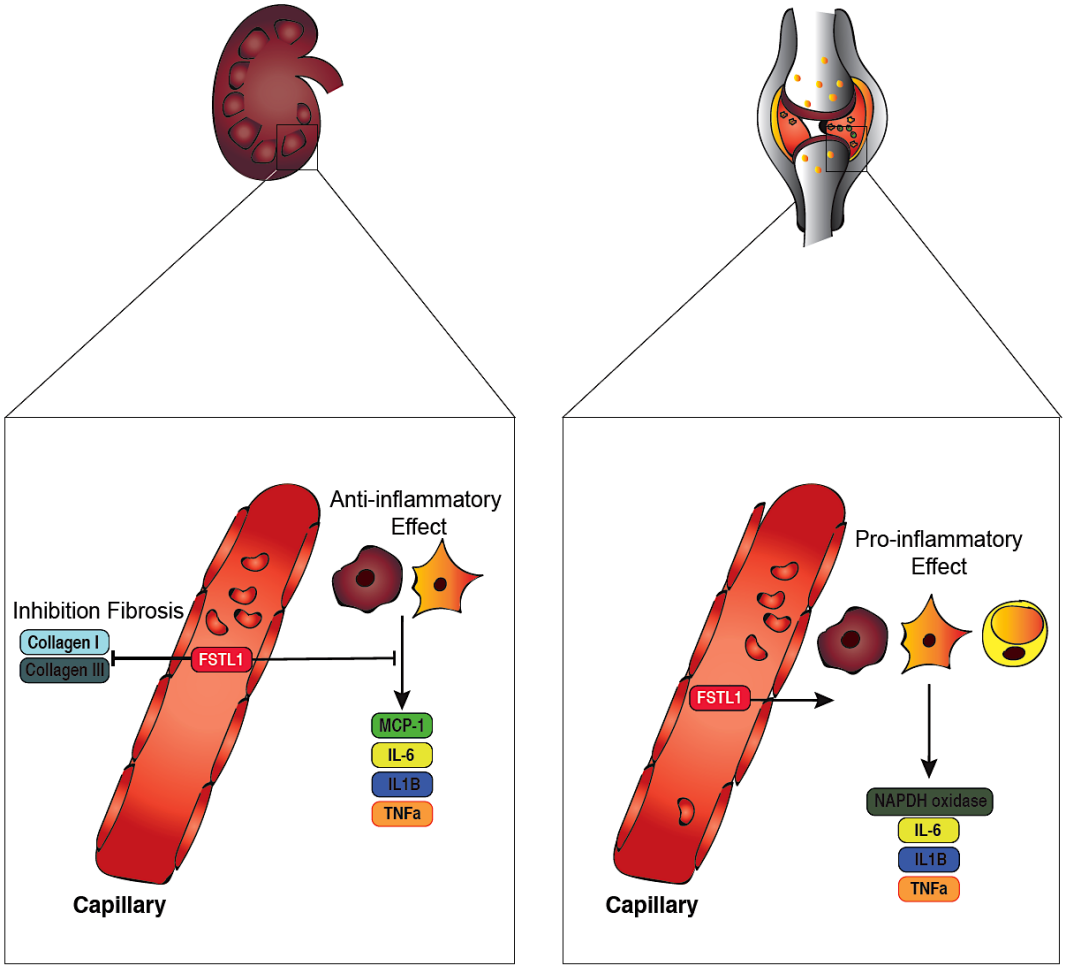

Fig. (2). The bifunctional role of FSTL1 in inflammation. In the kidney, FSTL1 has an anti-inflammatory effect by inhibiting fibrosis and macrophage and fibroblast cytokine secretion. In joints, FSTL1 can mediate the secretion of pro-inflammatory cytokines by macrophages, fibroblasts and adipocytes.

controls, and a relation was found between increased FSTL1 levels and the likelihood to develop coronary aneurysms [62]. Also, in patients with obesity (BMI ≥ 25 kg/m2), serum FSTL1 levels were significantly elevated compared to control patients. Furthermore, elevated FSTL1 mRNA levels were seen in subcutaneous and epididymal adipose tissue in a mouse model of obesity [63]. The pro-inflammatory capacity of FSTL1 is mediated by the expression of pro-inflammatory cytokines IL-6, IL-1β, TNFα, IFNγ-related genes [64], MCP-1 [65] and NF-κB signaling [66]. FSTL1 promotes IL-1β secretion by regulating the activity of the NLRP3 inflammasome both *in vitro* and *in vivo* [67]. The injection of FSTL1 in the paws of wild type mice caused swelling only in the presence of IFN-γ [64]. Not only exogenously administered FSTL1, but also endogenously expressed FSTL1 has a pro-inflammatory effect as shown in a mouse model of CIA treated with anti-FSTL1 antibodies. Here, amelioration of arthritis and reduced mRNA levels for IL-1β, IFN-γ, and CXCL10, which is a mediator in bone erosion in CIA, were observed [64, 68]. It appears most of the pro-inflammatory effects of FSTL1 are associated with its effect on arthritis. It is unclear whether this will also have implications for the therapeutic application of FSTL1 as a regenerative factor.

Other studies report the anti-inflammatory capacity of FSTL1. An ameliorating effect of recombinant human FSTL1 on arthritis in a mouse model of CIA has been described [69]. Treatment with FSTL1 prevented swelling of footpads and reduced the clinical score used to assess arthritis severity. Furthermore, FSTL1 was able to prevent cartilage breakdown and bone erosion by down regulating expression of c-fos, ets-2, Il-6, MMP-3, and MMP-9, genes that are involved in destructive joint inflammation [69-73]. In an *in vitro* model of neural inflammation in mouse astrocytes, FSTL1 attenuated the upregulation of pro-inflammatory cytokines after LPS treatment by suppressing the MAPK/p-ERK1/2 pathway [74]. Intravenous administration of FSTL1 in mice 4 weeks after subtotal nephrectomy ameliorated fibrosis and mice that received FSTL1 showed smaller glomerular area and fewer intraglomerular cells [75]. Lower mRNA levels of TNF-α, IL-6, IL-1β, MCP-1, NADPH oxidase components, connective tissue growth factor, TGF-β1, collagen I, and collagen III were found in the remaining kidney tissue of FSTL1 treated mice.

Mechanistically, the dual role of FSTL1 has been linked to pro-inflammatory processes via CD14 and TLR4 and to inhibition of tissue destruction via the downregulation of matrix metallopeptidase (MMPs) regulated via DIP2A, pAKT and up-regulation of FOS [76]. Thus, the capacity of FSTL1 to play either a pro- or anti-inflammatory role might stem from the various pathways through which FSTL1 is able to act.

As the inflammatory response is also important in multiple cardiac diseases and FSTL1 has been reported to have cardioprotective and regenerative effects, it is important to study whether FSTL1 treatment might have a pro-inflammatory effect in the heart. Still, the role of FSTL1 in cardiac inflammation remains largely unknown. Analysis of the expression of pro-inflammatory cytokines after FSTL1 in a mouse and pig model of I/R injury showed decreased levels of TNF-α and IL-6 [30]. Also, in cultured neonatal rat cardiomyocytes, lipopolysaccharide (LPS) stimulated expression of pro-inflammatory cytokines was decreased after FSTL1 treatment. FSTL1 supplementation to macrophages, which are abundantly present in the myocardium after cardiac I/R injury lead to an AMPK-dependent decrease in TNF-α and IL-6 expression after LPS or BMP-4 stimulation [30].

Furthermore, the effect of cardiac myocyte-derived FSTL1 on chronic kidney disease (CKD) was studied in a mouse model of subtotal nephrectomy comparing healthy mice to cardiac-specific FSTL1 knockout mice [75]. Significantly higher levels of pro-inflammatory cytokines TNF-α, IL-6, IL-1β, MCP-1, and NADPH oxidase components were expressed in FSTL1 knockout mice compared to control mice. It has been suggested that FSTL1 exerts anti-inflammatory effects via the inhibition of BMP-4 dependent inflammatory pathways [30] and pro-inflammatory effects via the activation of TLR4/MyD88/NF-kB and MAPK signaling pathways [77]. Thus, the capacity of FSTL1 to play either a pro- or anti-inflammatory role might stem from the various pathways through which FSTL1 is able to act. Another intriguing explanation of the ambiguous role of FSTL1 in inflammation and inflammatory diseases could be post-translational modification of FSTL1 [2].

1.10. Influence of Post-translational Modification on FSTL1 Function

Healthy epicardium expresses FSTL1, which is ceased after myocardial injury. As mentioned, FSTL1 can attenuate detrimental effects from myocardial injury by inducing cardiomyocyte proliferation, reducing apoptosis and inflammation, and promotion of revascularization [34, 41]. However, increasing FSTL1 circulating levels after myocardial infarction with FSTL1 from myocardial origin did not induce cardiomyocyte proliferation. Only FSTL1 from epicardial origin was found to be capable to induce a regenerative response [34]. Analysing and comparing biochemical properties of epicardial and myocardial FSTL1 revealed slower migration of myocardial FSTL1 in SDS polyacrylamide gel electrophoresis, representing increased molecular weight, potentially from post-translational modifications. Application of tunicamycin, an inhibitor and catalyst of reversion of N-linked glycosylation, abolished this difference in migration, suggesting hyperglycosylation as a cause of the observed high molecular weight FSTL1 myocardial isoform. Thus, it seems plausible that glycosylation and potentially other post-translational modifications play a role in modulating the abilities of FSTL1 to generate cardioprotective and regenerative responses.

In previous studies multiple glycosylated isoforms of FSTL1 with varying molecular weights have been detected [2, 4, 5]. Bacterially expressed recombinant FSTL1, primarily produced in *Escherichia coli,* is a hypoglycosylated isoform, whereas FSTL1 expressed in mammalian cells is extensively glycosylated. Glycosylated FSTL1 protected mouse embryonic stem cell-derived cardiomyocytes (mESC-CMs) from apoptosis following H_2O_2 application, although no effect on proliferation, indicating potential regenerative properties, was observed. In contrast, bacterially produced FSTL1 did stimulate proliferation of mESC-CMs but failed to attenuate H2O2-induced apoptosis [34]. FSTL1 produced in insect cells also inhibited apoptosis and inflammatory responses following ischemia/reperfusion injury, as shown in mouse and pig models [30]. However, when comparing FSTL1 expressed in mammalian cells, insect cells, and bacterial cells, no differences were found in the stimulation of fibroblast mobility, despite significant variations in the extent of glycosylation [78]. A recent study analysed whether ablation of the N glycosylation of the FSTL1 expressed in mammalian cells could increase the regenerative capacity of the human FSTL1 [79]. This study showed that a mutation in a single N glycosylation site (N180Q) of FSTL1 could trigger cardiomyocyte proliferation and cardiac regeneration in a mouse MI model. Based on these results, it is plausible that the upregulation of myocardial and downregulation of epicardial FSTL1 expression following MI alters distribution and ratios of hyper- and hypoglycosylated FSTL1 in the damaged heart.

Since several different isoforms and sources of FSTL1 have been used in previous studies and therefore results are difficult to compare across studies, Table **1** provides an overview of the specifications of previously used recombinant FSTL1 and the main results that have been found with each of these forms. However, precise mechanistic cues of post-translational modifications leading to either cardioprotective or regenerative capacities of FSTL1 still remain to be elucidated.

1.11. Method of Delivery to the Injured Heart

Previously described cardioprotective properties of FSTL1 *in vitro* and in small animal models are considered promising and raise the question which application method would be most suitable for FSTL1 to exhibit its effects in large animals and in humans. Systemic administration of FSTL1 is a conceivable option, however it is unlikely that it will have considerable beneficial effect as FSTL1 is already upregulated in response to various cardiac conditions, including ACS, ischemic cardiomyopathy, end-stage heart failure, and HFpEF. Intramyocardial injections with regenerative factors at the site of injury may potentially.

Table 1. Specifications of recombinant FSTL1 used in research on cardiac disease.

Parameter	Study	Origin FSTL1	Expression system	Tag	Supplier	*In Vitro/ Vivo*	Main Findings
Cardioprotection	Gorgens, 2013	Human	*E. coli*	6His at N-terminus	Aviscera Bioscience	*In vitro*	No alteration of Akt phosphorylation following insulin stimulation.
		Human	*E. coli*	His at N-terminus	GenWay		
	Maruyama, 2016	Human	HEK293	-	Proteos, Inc.	*In vitro*	Promotion of cardiac fibroblast mobility, however no significant differences between the isoforms.
		Human	*E. coli*	6His at N-terminus	Aviscera Bioscience		
		Mouse	Mouse cell line	6His at C-terminus	R&D Systems		
		Mouse	Insect Sf9 cell	FLAG at C-terminus	Walsh Laboratory		
	Ogura, 2012	Human	Insect Sf9 cells	FLAG at C-terminus	-	*In vitro/vivo*	Inhibition of apoptosis and inflammatory responses following cardiac ischemia/ reperfusion injury both *in vitro* and *in vivo*.
	Oshima, 2008	Mouse	HEK293	V5 epitope at C-terminus	-	*In vitro/vivo*	Reduction of apoptosis of neonatal rat cardiomyocytes after ischemia/reperfusion injury by upregulation of Akt and ERK signaling pathways; reduction of myocardial infarct size and apoptosis following myocardial infarction in mice.
	Ouchi, 2010	Mouse	Insect Sf9 cells	FLAG at C-terminus	-	*In vitro*	Enhanced survival, mobility, and differentiation into network structures in endothelial cells; reduced ischemia/reperfusion-induced apoptosis and stimulation of Akt phosphorylation in cardiomyocytes; effects attenuated by DIP2A.
	Zhang, 2017	Human FSTL1	Mouse myeloma cell line, NS0-derived		R&D Systems	*In vivo*	Decreased right ventricular systolic pressure and right ventricular hypertrophy index in mice with hypoxia-induced pulmonary hypertension.
Modulating cardiac hypertrophy	Tanaka, 2016	Human	Sf9 cells	FLAG at N-terminus	-	*In vivo*	Decrease of aldosterone-induced cardiomyocyte hypertrophy and diastolic dysfunction in mice with heart failure with preserved ejection fraction (HFpEF).
Cardiomyocyte proliferation	Wei, 2015	Human FSTL1	*E. coli*	6His at N-terminus	Aviscera Bioscience	*In vitro/in vivo*	Stimulated proliferation of mouse embryonic stem cell-derived cardiomyocytes but did not protect from H_2O_2-induced apoptosis; faster migration through SDS polyacrylamide gels compared to mammalian-expressed FSTL1; stimulated cardiomyocyte proliferation, cardiac regeneration and survival following ischemia/reperfusion injury in mice and swine models.
		Human FSTL1	Mouse myeloma cell line, NS0-derived	Asp and 10His at C-terminus	R&D Systems	*In vitro*	Inhibited H_2O_2-induced apoptosis in mouse embryonic stem cell-derived cardiomyocytes but did not have a proliferative effect; slower migration through SDS polyacrylamide gels compared to bacterial-expressed FSTL1.

(Table 1) Contd….

Parameter	Study	Origin FSTL1	Expression system	Tag	Supplier	*In Vitro/ Vivo*	Main Findings
Cardiorenal communication	Hayakawa, 2015	Human	Insect Sf9 cells	FLAG at C-terminus	-	*In vitro*	Decreased expression of pro-inflammatory cytokines and increased phosphorylation of AMP-activated protein kinase in human mesangial cells.
Endothelial cells and revascularization	Miyabe, 2014	Human	Insect Sf9 cells	FLAG at C-terminus	-	*In vitro*	Decreased proliferation and migration of human aortic smooth muscle cells by activation of AMPK.
	Wu, 2015	Human FSTL1	Mouse myeloma cell line, NS0-derived	Asp and 10His at C-terminus	R&D Systems	*In vitro*	Attenuated hemin-induced differentiation and survival of erythroid cells; regulated TGF-β and BMP expression.
	Xi, 2016	Human FSTL1	*E. coli*	21His at N-terminus	ProSpec	*In vivo*	Stimulated angiogenesis and improved systolic cardiac function after myocardial infarction in rats.
Biomarker	Widera, 2012	Mouse FSTL1	Sf9 insect cells	-	-	*In vitro*	Increased production of GDF 15 mRNA in adult mouse cardiomyocytes.
	Widera, 2009	Human FSTL1	NSO mouse myeloma cells	-	R&D Systems	*In vitro*	Used for calibration to calculate serum FSTL1 concentrations.
Chemical properties	Hambrock, 2004	Mouse	HEK293	His-Myc at N-terminus	-	*In vitro*	No structural changes following calcium addition or depletion.
	Li, 2013	Murine	*Drosophila* S2 cells	6His at C-terminus	-	*In vitro*	Inhibition of BMP4-induced phosphorylation of Smad1/5/8 in Mv1Lu cells.
	Tanaka, 2010	Human	COS-7 cells	FLAG	-	*In vitro*	DIP2A may be a receptor for FSTL1; FSTL1 binds to DIP2A, CD14, and other proteins of the TGF-β superfamily.

In summary, FSTL1 expression and function has been shown to be closely linked to cardiac disease, both as a marker, and increasingly as a potential therapeutic compound or target. It is important to determine the mechanism and extent of the specific pro- and anti-inflammatory effects when FSTL1 is considered as a potential therapeutic agent or target to treat cardiovascular diseases. Finally, the method of application of FSTL1 in the context of cardiovascular disease will also very likely play a role in how inflammatory responses turn out. Characterization of the effect of FSTL1 on inflammatory cell infiltration and activation in the heart is essential before the therapeutic application can be considered.

CONCLUSION

FSTL1 is a cardiokine with multiple implications in cellular processes in the heart, particularly in response to cardiac injury. An increasing body of evidence indicates that FSTL1 may attenuate I/R injury by inhibiting apoptosis and stimulating proliferation and revascularization, suggesting a potential role in regenerative therapy for heart failure patients. Furthermore, a protective role of FSTL1 has been described in cardiac hypertrophy and pulmonary hypertension. Effects and function of FSTL1 in the context of inflammation remain ambiguous and require further research, especially in acute and chronic cardiovascular disease. The spatio-temporal organization of FSTL1 expression, its localization and onset after induction of damage, as well as post-translational modifications of FSTL1, mostly in terms of glycolysation, have been shown to be critical parameters of activity. The hypoglycosylated epicardial FSTL1, diminished upon myocardial injury, holds most potential in exerting cardioprotective and regenerative effects. To achieve this, epicardial reconstitution of FSTL1 following I/R injury may be preferred, despite the risks associated with a potentially invasive procedure.

In conclusion, FSTL1 exhibits regenerative and tissue-protective features making it a promising candidate for novel approaches to treat cardiovascular disease, while mechanistic details need further research before advancing to therapeutic applications.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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