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ORIGINAL ARTICLE

Wnt5a is elevated in heart failure and affects cardiac fibroblast function

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Abstract

Wnt signaling is dysregulated in heart failure (HF) and may promote cardiac hypertrophy, fibrosis, and inflammation. Blocking the Wnt ligand Wnt5a prevents HF in animal models. However, the role of Wnt5a in human HF and its functions in cardiac cells remain unclear. Here, we investigated Wnt5a regulation in HF patients and its effects on primary mouse and human cardiac fibroblasts. Serum Wnt5a was elevated in HF patients and associated with hemodynamic, neurohormonal, and clinical measures of disease severity. In failing human hearts, Wnt5a protein correlated with interleukin (IL)-6 and tissue inhibitor of metalloproteinase (TIMP)-1. Wnt5a messenger RNA (mRNA) levels were markedly upregulated in failing myocardium and both mRNA and protein levels declined following left ventricular assist device therapy. In primary mouse and human cardiac fibroblasts, recombinant Wnt5a dose-dependently upregulated mRNA and protein release of IL-6 and TIMP-1. Wnt5a did not affect β-catenin levels, but activated extracellular signal-regulated kinase 1/2 (ERK1/2) signaling. Importantly, inhibition of ERK1/2 activation attenuated Wnt5a-induced release of IL-6 and TIMP-1. In conclusion, our results show that Wnt5a is elevated in the serum and myocardium of HF patients and is associated with measures of progressive HF. Wnt5a induces IL-6 and TIMP-1 in cardiac fibroblasts, which might promote myocardial inflammation and fibrosis, and thereby contribute to HF progression.

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Key messages

- Wnt5a is elevated in serum and myocardium of HF patients and is associated with measures of progressive HF.
- In cardiac fibroblasts, Wnt5a upregulates interleukin (IL)-6 and tissue inhibitor of metalloproteinase (TIMP)-1 through the ERK pathway.
- Wnt5a-mediated effects might promote myocardial inflammation and fibrosis, and thereby contribute to HF progression.

Keywords Wnt5a · Wnt signaling · Heart failure · Il-6 · TIMP-1 · ERK

Introduction

Heart failure (HF) is a major cause of morbidity and mortality worldwide with increasing incidence due to aging population [1]. Pathological stimuli induce a series of alterations in the heart, referred to as myocardial remodeling, where mechanically stressed cardiac cells secrete humoral factors that alter myocardial structure and function, promote cardiac hypertrophy, fibrosis, and inflammation, leading to development and progression of HF [2]. Initially, a useful compensatory response, myocardial remodeling, is harmful in the long run.

The Wnt pathway is involved in myocardial remodeling [3–5] and consists of multiple ligands and antagonists which transduce signals through a canonical, β-catenin-dependent pathway and several non-canonical ones, including the planar cell polarity, Wnt/Ca^{2+} [4, 6], and others, less studied, such as the extracellular-regulated kinase 1/2 (ERK1/2) cascade [7]. Outcomes of Wnt signaling depend on receptor context, cell, and tissue type [6, 8, 9]. The non-canonical ligand Wnt5a has emerged as an important player in cardiac disease, inflammatory disorders, and cancer [9, 10]. Wnt5a may induce cardiomyocyte hypertrophy while its inhibition attenuates hypertrophy [11] and HF development [12, 13]. Furthermore, Wnt5a promotes inflammation and fibrosis in multiple tissues including the lung [14, 15]. Recently, Wnt5a was implicated in fibrosis in the neonatal heart after cryoinjury [16].

The role of Wnt5a in human HF is still largely unknown. Cardiac fibroblasts (CFs) play a major role in myocardial inflammation and ECM remodeling, but the effects of Wnt5a on these cells have not been clarified. To further elucidate the role of Wnt5a in HF, we evaluated (i) the expression of Wnt5a in serum and myocardium of HF patients and associations with measures of HF severity and adverse outcome, (ii) Wnt5a-mediated effects on

expression of fibrotic and inflammatory markers as well as activation of β-catenin and ERK signaling in adult mouse CFs, and (iii) confirmed the main findings in primary human CFs.

Materials and methods

Patients and study procedures

The study population consisted of 155 consecutively recruited patients with stable HF (>6 months) and reduced left ventricular (LV) ejection fraction (LVEF) of the New York Heart Association (NYHA) functional classes II–IV. The underlying cause of HF (Table 1) was based on medical history, coronary angiography, and echocardiographic examination. Patients with acute coronary syndromes within the last 6 months or with significant concomitant diseases (e.g., infection, malignancy, or autoimmune disorders) were excluded. Twenty-five age- and sex-matched healthy individuals (19 males, mean ± SD age 58.2 ± 7.8 years) served as controls (serum cohort). Echocardiography and right-sided heart catheterization was performed as described [17].

Blood sampling and biochemical analyses

Details on analysis of Wnt5a, interleukin-6 (IL-6), tissue inhibitor of metalloproteinase 1 (TIMP-1), tumor necrosis factor (TNF), chemokine (C-X-C motif) ligand 2 (CXCL2), secreted frizzled-related protein 3 (sFRP3), C-reactive protein (CRP), and N-terminal pro-brain natriuretic peptide (NT-proBNP) are given in the Supplementary Material.

Tissue sampling from human myocardium

LV myocardial biopsies were collected from (i) still-beating hearts immediately on explantation from patients with end-stage HF undergoing heart transplantation (Table 1), (ii) patients with structurally normal hearts (controls), and (iii) 18 patients with advanced HF at the time of implantation and at the time of removal (heart transplantation) of a continuous-flow LV assist device (LVAD) as detailed in the Supplementary Material.

Immunohistochemistry

Immunohistochemical staining of Wnt5a protein expression in failing human hearts obtained from HF patients after

Table 1 Characteristics of patients with stable HF (n = 155) and end-stage HF who underwent heart transplantation (n = 82)

Variable	Serum cohort (n = 155)	Tissue cohort (n = 82)
Age, years	57 ± 11	48 ± 16
Male gender, %	83	73
Body mass index, kg/m^2	26.7 ± 5.6	25.9 ± 4.3
Coronary artery disease/dilated cardiomyopathy/other, %	47/48/5	68/31/0
NYHA class II/III/IV, %	26/48/26	1/58/41
Duration HF, years	2 (1, 7)	6 (1, 11)
Smokers, %	49	39/51/10
Heart rate (beats/min)	77 ± 15	79 ± 15
Atrial fibrillation, %	16	17
Hypertension, %	17	9
Diabetes mellitus, %	15	18
Hemoglobin, g/dl	14.0 ± 1.7	12.9 ± 1.9
Creatinine, mmol/l	86 ± 21	108 ± 38
Leukocytes	7.7 ± 2.2	9.0 ± 4.4
Platelets (10^9/L)	269 (228, 316)	236 (203,292)
Cholesterol, mmol/l	4.8 ± 1.2	4.2 ± 2.4
Medication, %		
Loop diuretics	63	86
Aldosterone antagonist	39	49
Angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker	74	90
β-blocker	73	83
Statins	42	36

explantation was performed as detailed in the Supplementary Material.

Cardiac cell isolation and culture

Mouse cardiac cell separation, CF isolation, and culturing was performed as previously described [18, 19] and detailed in the Supplementary Material. Primary human CFs isolated from the ventricles of an adult heart were purchased from PromoCell (Heidelberg, Germany), grown and cultured as described in the Supplementary Material.

RNA extraction and reverse-transcription qPCR

Total RNA was extracted from human myocardial tissue and CF cell pellet and qPCR performed as described in the Supplementary Material.

Immunoblot analysis

Total protein was isolated from CF pellet and immunoblot analysis was performed on protein homogenates as described in the Supplementary Material.

Statistics

Statistical differences between groups were tested using Student's t test or the Mann–Whitney U test depending on distribution, and χ^2 test for categorical variables. One-way and two-way ANOVA or Kruskal–Wallis test was used to compare greater than two groups. Association between Wnt5a tertiles and all-cause mortality or heart transplantation was evaluated by Kaplan–Meier analysis with log-rank test. Associations between Wnt5a levels and clinical parameters as well as gene expression in human myocardium were assessed with the Spearman correlation. Two-sided probability values were considered significant at $p < 0.05$.

Results

Serum Wnt5a is elevated and associated with disease severity in HF patients

HF patients (Table 1) had elevated Wnt5a levels compared with age- and sex-matched controls (Fig. 1a) with higher levels according to clinical disease severity (NYHA, Fig. 1b), but no relation to etiology ($p = 0.64$). Among hemodynamic measures (Table 2), Wnt5a positively correlated with E/A ratio as a marker of diastolic dysfunction (Fig. 1c), pulmonary artery systolic pressure (PASP), pulmonary artery resistance, and negatively correlated with cardiac output, but not LVEF (Table 2). Patients with PASP >40 mmHg, indicating pulmonary hypertension, had increased Wnt5a levels (Fig. 1d). Wnt5a correlated modestly with CRP and NT-proBNP, reflecting systemic inflammation and myocardial wall stress, respectively (Table 2).

The ratio between Wnt5a and its antagonist sFRP3 is associated with transplant-free survival in HF patients

During a median follow-up of 5.6 (range 0.1–7.8) years, 39 (26%) patients died and an additional 14 (9%) patients underwent heart transplantation (composite outcome). The Kaplan–Meier analysis for the composite of all-cause mortality or heart transplantation based on Wnt5a tertiles was not significant, but suggested a non-linear association with outcome (Fig. 1e). Tertile 3 had a significantly higher outcome rate vs. tertile 1 and 2 combined ($p = 0.025$).

We have previously analyzed circulating sFRP3, an antagonist of Wnt5a, in this patient cohort [20] and using the Wnt5a/sFRP3 ratio as a surrogate for Wnt5a activity revealed a worse outcome for patients in the highest tertile (Fig. 1f). By Cox regression analysis, the hazard ratio and 95% confidence intervals, associated with a 1-SD increase in Wnt5a/sFRP3 ratio, was 2.52 (1.44–4.42), which remained significant when
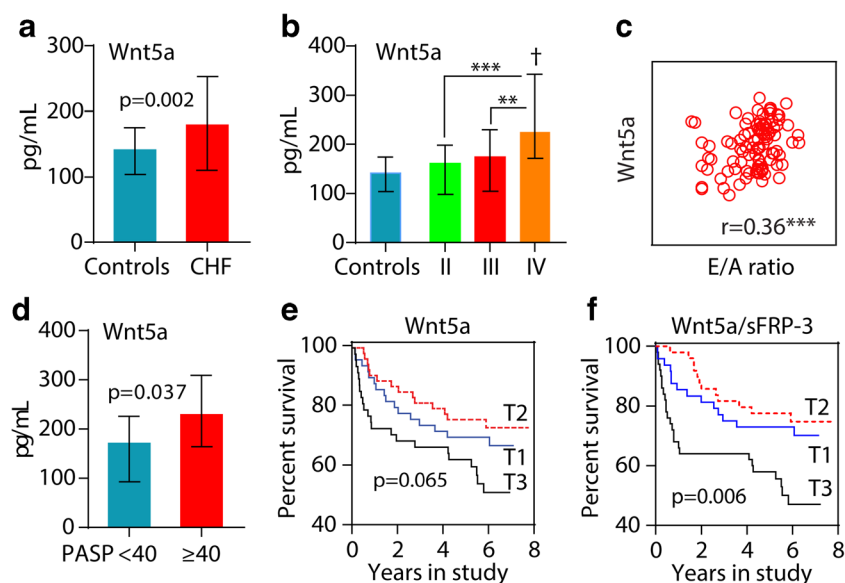
Fig. 1 Serum Wnt5a is elevated and associated with disease severity in heart failure patients. **a** Serum Wnt5a levels in HF patients ($n = 155$) compared to healthy controls ($n = 25$). **b** Association between Wnt5a levels and New York Heart Association (NYHA) class. **c** Association between serum Wnt5a levels and E/A ratio. **d** Serum Wnt5a in patients with pulmonary hypertension (PASP ≥40 mmHg) and without it (PASP <40 mmHg). **e** Kaplan–Meier curves showing adverse outcome according to Wnt5a tertiles and **f** tertiles of Wnt5a/sFRP3 ratio. Data are presented as median and 25th and 75th percentile. $**p < 0.01$, $***p < 0.001$, $†p < 0.05$ vs controls. *HF* heart failure, *PASP* pulmonary artery systolic pressure, *sFRP3* secreted frizzled-related protein 3

adjusting for age, gender, eGFR, NYHA, and NT-proBNP: 1.93 (1.07–3.51) $p = 0.030$.

Wnt5a is increased in failing human hearts

Immunohistochemical staining demonstrated abundant Wnt5a protein expression in failing human hearts (Fig. 2a) and LV Wnt5a messenger RNA (mRNA) levels were elevated in end-stage HF compared to non-failing hearts (Fig. 2b), with no difference according to etiology ($p = 0.78$). Moreover,

Wnt5a mRNA and protein expression decreased following LVAD treatment (Fig. 2c), suggesting that Wnt5a may be involved in myocardial remodeling. In addition, BNP mRNA levels, an established marker of wall-stress, also declined after LVAD therapy (Fig. 2d). Furthermore, when dichotomizing the change in Wnt5a mRNA during LVAD, patients with a large decrease in LV Wnt5a expression had a more pronounced decrease in BNP mRNA levels (Fig. 2e). Wnt5a LV mRNA and protein correlated negatively with LVEF and positively with NT-proBNP (Table 2). Wnt5a

Table 2 Associations between serum or myocardial Wnt5a levels and C-reactive protein, NT-proBNP, and hemodynamic variables

Variable	Serum cohort			Tissue cohort			
	n	Value	r	n (mRNA/protein)	Value	r (mRNA)	r (protein)
E/A ratio[a]	100	1.6 (0.8, 2.8)	0.36***	50/46	2.3 (1.6, 3.0)	0.16	0.06
Pulmonary artery systolic pressure (mmHg)	88	42 ± 16	0.22*	74/64	41 ± 14	0.27*	0.16
Pulmonary artery resistance (Wood unit m^2)	88	2.3 (1.6, 3.3)	0.30**	74/64	2.2 (1.6, 3.0)	0.29*	0.22
Pulmonary capillary wedge pressure (mmHg)	88	18 ± 8	0.19	74/64	18 ± 8	0.20	0.17
LV ejection fraction (%)	141	28 (20, 35)	−0.12	69/60	20 (15, 23)	−0.30*	−0.28*
Cardiac output (l/min)	137	4.2 (3.5, 4.9)	−0.27*	67/64	4.3 (3.3, 5.2)	−0.08	−0.19
C-reactive protein (mg/l)	155	3.9 (1.9, 12.0)	0.20*	76/65	5.6 (2.7, 14.0)	0.19	0.13
NT-proBNP (pmol/l)	155	237 (93, 440)	0.22*	76/65	308 (158, 627)	0.24*	0.45**

Data are given as mean ± SD or median (25th/75th) percentile depending on distribution

n indicates the number of observations

[a] Excluding patients with atrial fibrillation
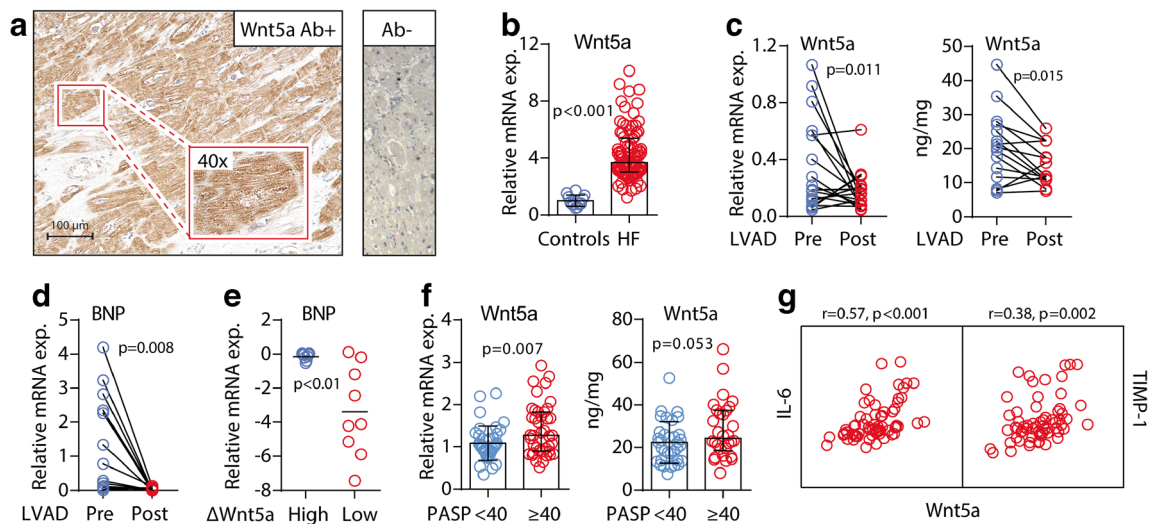
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Fig. 2 Increased Wnt5a expression in failing human myocardium. mRNA is expressed relative to GAPDH and protein is expressed ng Wnt5a/mg total protein. **a** Representative immunohistochemical staining of Wnt5a in failing human hearts (*n* = 6). **b** Relative mRNA expression of Wnt5a in myocardium of patients with end-stage HF (*n* = 82) compared to non-failing myocardium (*n* = 12). **c** Wnt5a mRNA and protein levels and **d** BNP mRNA levels in myocardium of HF patients before (pre) and after (post) LVAD therapy (*n* = 18). **e** The change in Wnt5a mRNA levels in relation to the change in BNP levels in patients on LVAD therapy. **f** Myocardial Wnt5a mRNA and protein levels in patients with pulmonary hypertension (PASP ≥40 mmHg) and without it (PASP <40 mmHg). **g** Correlations between myocardial Wnt5a and IL-6 and TIMP-1 levels. *Ab* antibody, *BNP* brain natriuretic peptide, *HF* heart failure, *IL-6* interleukin-6, *LV* left ventricular, *LVAD* LV assist device, *PASP* pulmonary artery systolic pressure, *TIMP-1* tissue inhibitor of metalloproteinase 1

mRNA was positively correlated with PASP and pulmonary artery resistance (Table 2). Patients with pulmonary hypertension (PASP >40 mmHg) had increased myocardial Wnt5a mRNA levels with a similar trend for Wnt5a protein (Fig. 2f). Furthermore, LV Wnt5a protein levels correlated with IL-6 and TIMP-1 (Fig. 2g). In contrast, the canonical Wnt ligands Wnt3a and Wnt8a were barely detectable in myocardial tissue from HF patients (data not shown).

Wnt5a promotes IL-6 and TIMP-1 release from mouse cardiac fibroblasts

As shown in Fig. 3a, the main cellular source of Wnt5a in mouse heart appears to be CFs, with ninefold higher expression compared to levels in cardiomyocytes. We next investigated if Wnt5a could induce production of relevant cytokines in these cells and found that Wnt5a increased protein release of IL-6 in the conditioned medium in a dose-dependent manner, while no effects were found with heat-inactivated Wnt5a (Fig. 3b). In addition to being inflammatory cells, CFs play a major role in ECM remodeling and fibrosis and as shown in Fig. 3c, a similar dose response as observed for IL-6 was found for the fibrotic marker TIMP-1.

Time course experiments using 30 nM Wnt5a revealed a transient response with a maximum increase in IL-6 and TIMP-1 mRNA expression at 5 h (Fig. 3d) and 8 h (Fig. 3e), respectively, while a linear increase in protein release was observed up to 24 h (Fig. 3f, g). Other inflammatory cytokines were not affected (Online Resource 1). There were no effects of Wnt5a

treatment on the mRNA expression of other fibrotic markers, α smooth muscle actin (αSMA), matrix metalloproteinase (MMP)-14 or collagen type 1, except for a minor increase in αSMA at 12 h, and modest decrease in mRNA expression was observed for MMP-2, TIMP-2, and TIMP-4 toward the end of the experiment (Online Resource 2). Finally, Wnt5a may also play a role in angiogenesis [21] but we found no effects of Wnt5a on angiogenic markers relevant for myocardial remodeling and regulation of capillary density, i.e., VEGF, SDG1, and SPRED1 (Online Resource 3).

Wnt5a does not affect β-catenin, but activates ERK1/2 signaling

Next, we investigated signaling pathways that could be involved in Wnt5a-induced IL-6 and TIMP-1 release. As shown in Fig. 4a and Online Resource 4, recombinant Wnt5a did not affect β-catenin protein levels, but reduced the expression of the canonical Wnt pathway target genes Axin2, cFos, and cJun (Fig. 4b), possibly through inhibition of canonical Wnt signaling at the level of T cell factor transcription [8].

The ERK1/2 pathway has previously been demonstrated to be involved in IL-6 and TIMP-1 upregulation [22, 23] and Wnt5a markedly increased ERK1/2 phosphorylation (Fig. 4c and Online Resource 4). Furthermore, blocking ERK1/2 activation with the MEK inhibitor PD98059 suppressed Wnt5a-induced mRNA expression and protein release of IL-6 and TIMP-1 (Fig. 4d, e), indicating that Wnt5a-induced IL-6 and TIMP-1 release is dependent on ERK1/2.
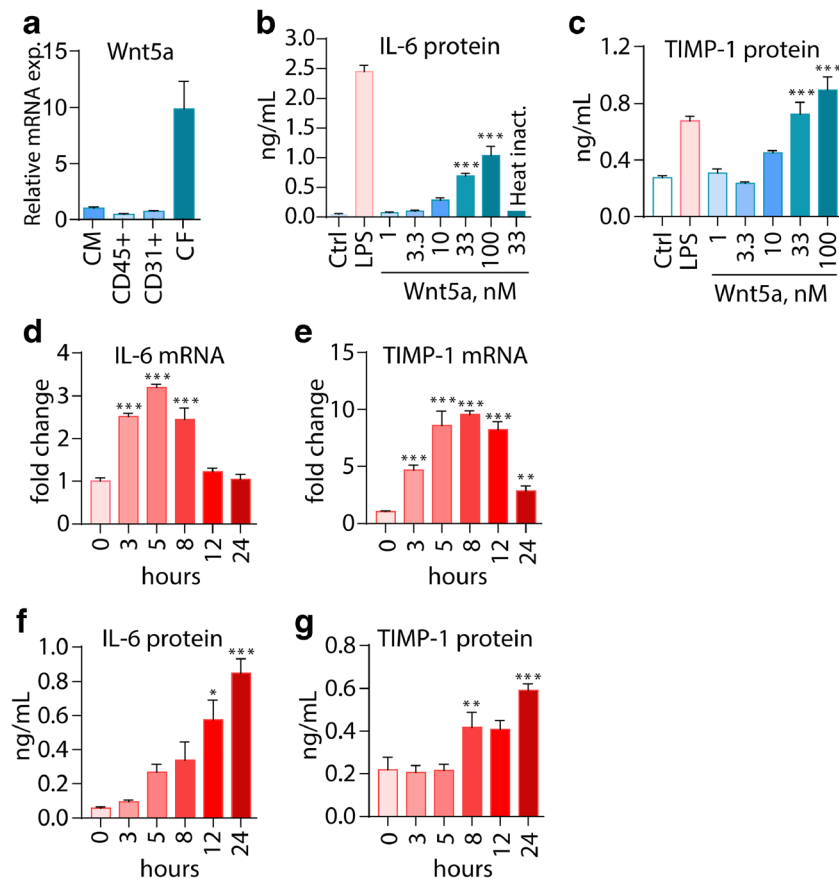
Fig. 3 Wnt5a promotes IL-6 and TIMP-1 release from mouse cardiac fibroblasts. mRNA is expressed relative to GAPDH. **a** Relative Wnt5a mRNA expression in fractionated mouse heart cells: cardiomyocytes (*CM*), leukocytes (CD45^{+}), endothelial cells (CD31+), and cardiac fibroblast (CF)-rich fraction. **b** Protein release of IL-6 and **c** TIMP-1 after treatment with increasing doses of Wnt5a for 24 h. **d** Relative mRNA expression of IL-6 and **e** TIMP-1 after treatment with 30 nM Wnt5a at different time points. **f** Protein release of IL-6 and **g** TIMP-1 after treatment with 30 nM Wnt5a at different time points. Data are presented as mean ± SEM of three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs control. *IL-6* interleukin-6, *LPS* lipopolysaccharide, *TIMP-1* tissue inhibitor of metalloproteinase 1

Wnt5a increases sFRP3 release from mouse cardiac fibroblasts

The Wnt antagonist sFRP3 may bind and antagonize the effects of Wnt5a [24] and is abundantly expressed in cardiac cells and in particular CFs [20]. Recombinant Wnt5a increased sFRP3 protein release in a dose- and time-dependent manner (Fig. 5a, b), but did not alter sFRP3 mRNA expression (Fig. 5c). Moreover, the MEK inhibitor had no effect on sFRP3 protein release (Fig. 5d), suggesting that ERK1/2 is not required for Wnt5a-induced sFRP3 release.

Wnt5a promotes IL-6 and TIMP-1 release through the ERK1/2 signaling in human cardiac fibroblasts

We next aimed to confirm our main findings from primary mouse CFs in primary human CFs. We found a similar pattern of Wnt5a responses in primary human CFs with an increase in mRNA expression and release of IL-6 and TIMP-1 in a dose-and time-dependent manner (Fig. 6a–f). Moreover, inhibition of ERK1/2 activation suppressed Wnt5a-induced IL-6 and TIMP-1 mRNA levels and protein release, although the effect on IL-6 protein levels did not reach statistical significance (Fig. 6g, h).

Discussion

Several lines of evidence support a critical role for dysregulated Wnt signaling in the pathogenesis of cardiac disease [3–5]. Herein, we provide clinical support by demonstrating elevated systemic and myocardial Wnt5a in HF patients, associated with hemodynamic, neurohormonal, and clinical measures of disease severity. We show that myocardial Wnt5a protein correlated with IL-6 and TIMP-1, markers of inflammation and fibrosis. Furthermore, Wnt5a induced IL-6 and TIMP-1 through ERK1/2 activation in vitro. Our findings suggest that Wnt5a-induced non-canonical Wnt signaling may contribute to HF progression.
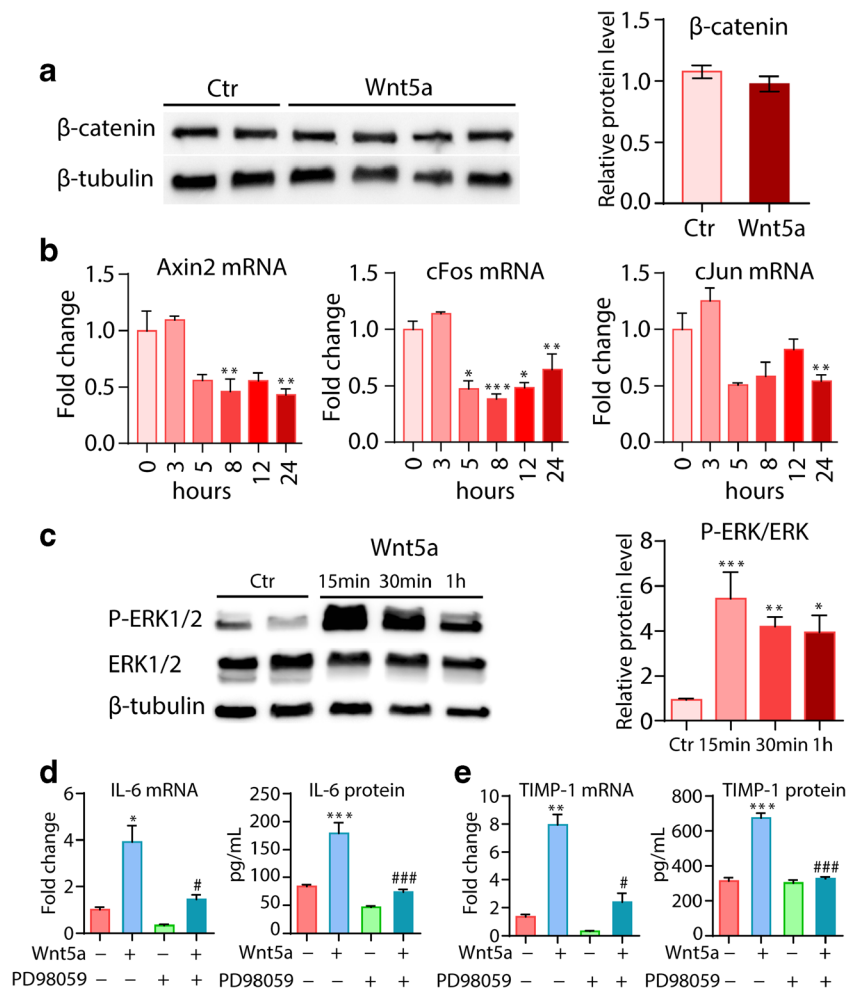
Fig. 4 Wnt5a activates ERK1/2 in mouse cardiac fibroblasts. mRNA is expressed relative to GAPDH. **a** Representative immunoblot and quantification of β-catenin and β-tubulin (loading control) in CFs treated with 30 nM Wnt5a for 3 h. **b** Relative mRNA expression of β-catenin target genes in CFs treated with 30 nM Wnt5a. **c** Representative immunoblot and quantification of p-ERK1/2, total ERK1/2, and β-tubulin (loading control) in CFs treated with 30 nM Wnt5a. **d** Relative mRNA expression and protein release of IL-6 and **e** TIMP-1 from CFs treated with recombinant Wnt5a, MEK inhibitor PD98059, or a combination of both for 8 h. Data are shown as mean ± SEM of three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs control; #$p < 0.05$, ###$p < 0.001$ vs Wnt5a. *CFs* cardiac fibroblasts, *ERK1/2* extracellular signal-regulated kinase 1/2, *IL-6* interleukin-6, *MEK* mitogen-activated protein kinase kinase, *TIMP-1* tissue inhibitor of metalloproteinase 1

Experimental studies implicate canonical and non-canonical Wnt pathway activation in processes that lead to HF development, such as cardiac hypertrophy, fibrosis, myocardial remodeling, and vascular calcification [3–5]. In contrast, the canonical Wnt3a and Wnt8a ligands were scarcely expressed in our myocardial tissue from end-stage HF patients. However, our findings, showing upregulated systemic and myocardial levels of Wnt5a that were associated with measures of disease severity, support a role for the non-canonical Wnt activation in clinical HF.

Several reports have identified a critical role of Wnt signaling in pulmonary hypertension [25, 26], pulmonary remodeling, and fibrosis [14, 15]. Long-standing pulmonary arterial pressure overload may eventually lead to RV failure, characterized by increased filling pressures, diastolic dysfunction [27], and extensive remodeling in the pulmonary vasculature [28]. Our finding that systemic and myocardial Wnt5a were elevated in pulmonary hypertension may suggest a role for Wnt5a in RV failure and the progression to end-stage HF. Moreover, circulating Wnt5a was associated with elevated E/A ratio, a measure of diastolic dysfunction, which is often caused by heart stiffness and fibrosis [29]. Wnt5a is implicated in fibrosis of multiple organs [14, 15, 30], and the association between the E/A ratio and serum Wnt5a could reflect increased ECM turnover and release of Wnt5a into the circulation. Furthermore, myocardial Wnt5a protein levels correlated with IL-6 and TIMP-1, which have an established pathophysiological role in HF. Both are elevated in HF patients, IL-6 increases collagen synthesis and causes myocardial fibrosis, concentric hypertrophy and diastolic dysfunction [31, 32], while TIMP-1 promotes cardiac fibrosis [33–35].
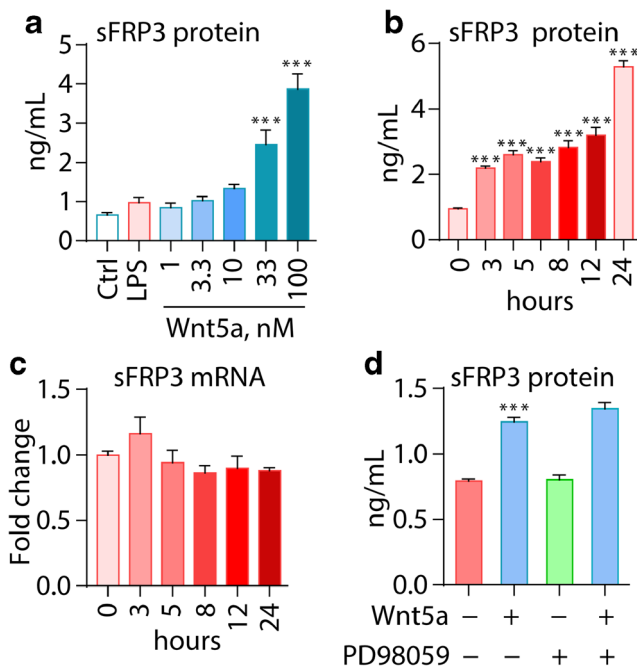
Fig. 5 Wnt5a increases sFRP3 release from mouse cardiac fibroblasts. mRNA is expressed relative to GAPDH. **a** sFRP3 release from CFs after treatment with different doses of Wnt5a or LPS. **b** sFRP3 release and **c** mRNA expression in CFs after treatment with 30 nM Wnt5a for different time points. **d** sFRP3 release from CFs treated with recombinant Wnt5a, MEK inhibitor PD98059, or a combination of both. Data are mean ± SEM of three independent experiments. ***$p < 0.001$. *CFs* cardiac fibroblasts, *sFRP3* secreted frizzled-related protein 3, *LPS* lipopolysaccharide, *MEK* mitogen-activated protein kinase kinase

CFs are among the most abundant cell types in the heart and have major roles in inflammation and ECM regulation by producing structural proteins (e.g., collagen), enzymes (i.e., MMPs), and inhibitors (i.e., TIMPs). A balanced regulation of these proteins is necessary for normal ECM homeostasis and alterations in their expression and activity can lead to pathological remodeling and fibrosis [36]. Herein, we show that Wnt5a was most abundantly expressed in mouse CFs and enhanced the mRNA expression and release of TIMP-1 through ERK1/2 signaling in primary mouse and human CFs. ERK1/2 is known to contribute to cardiac fibrosis by stimulating collagen synthesis and TIMP-1 expression [22, 37], while ERK1/2 inhibition ameliorates fibrosis and cardiac dysfunction [37]. Thus, our findings suggest that Wnt5a could be involved in HF progression by promoting cardiac fibrosis through TIMP-1 and ERK1/2. There is limited data on Wnt5a effects on ECM markers in CFs. No effects of Wnt5a overexpression on TIMP-1 were observed in C2C12 myoblasts [38], while Wnt5a increased ECM production by elevating fibronectin and α5-integrin levels in lung fibroblasts [15] and MMP-3 activity in rat-dental fibroblast-like cells [39]; Wnt5a may activate ERK1/2 leading to the induction of IL-6 in bone marrow stromal cells [23] and CCL2 in neutrophils [40]. Similar to TIMP-1, Wnt5a dose dependently enhanced

IL-6 expression and release from mouse and human CFs, suggesting that Wnt5a may modulate inflammatory responses in cardiac cells in an ERK-dependent manner. Wnt5a has been shown to promote HF development by stimulating hypertrophic responses in cardiomyocytes [11]. Our study suggests that Wnt5a could also promote HF progression by inducing inflammatory and pro-fibrotic responses in CFs, which could contribute to systolic and diastolic dysfunction. However, experimental models are needed to evaluate the precise role of Wnt5a and its therapeutic potential in HF.

Little is known about the regulation of Wnt5a in CFs. In lung fibroblasts, Wnt5a is induced by transforming growth factor β1, a key modulator of ECM remodeling and fibrosis [14]. The transcription factors NF-kappaB and MAP kinases may also have distinct roles in determining the activity of Wnt5a promoters [41]. Increasing evidence suggests that microRNAs also may regulate Wnt5a [42–44], e.g., miR-223 upregulates Wnt5a expression [42]. Interestingly, DNA damage decreases miR223 [45] and oxidative stress and DNA damage have been linked to HF [46]. Thus, a decrease in miR-223 due to DNA damage could potentially cause Wnt5a upregulation in HF patients. In addition, miR126 and inflammation have been linked to decreased angiogenesis in HF in particular in the context of RV remodeling and pulmonary hypertension [47, 48], but we found no effects of Wnt5a on relevant angiogenic markers, although this should be more thoroughly investigated in forthcoming studies.

We have recently demonstrated increased circulating and myocardial expression of Wnt antagonist sFRP3 in HF, with high levels in CFs [20, 49]. Our finding that sFRP3 is released from mouse CFs upon Wnt5a treatment may suggest that enhanced sFRP3 in HF could reflect active Wnt signaling and represent a negative feedback loop to limit Wnt pathway activity. A more prominent effect of a high Wnt5a/sFRP3 ratio on adverse outcome, however, could indicate that sFRP3 is unable to adequately antagonize Wnt5a in HF patients with end-stage disease.

Limitations in our study include that samples were obtained from HF patients with reduced ejection fraction and our results may therefore not apply to patients with preserved systolic function. Also, we did not have healthy myocardial tissue for protein analysis and comparison with failing myocardium.

Conclusions

Our study demonstrates that HF patients are characterized by increased systemic and myocardial Wnt5a levels that correlate with indices of disease progression as well as myocardial IL-6 and TIMP-1 protein levels. In primary mouse and human CFs, Wnt5a induces IL-6 and TIMP-1 production through the non-canonical ERK1/2 pathway. These Wnt5a-mediated effects
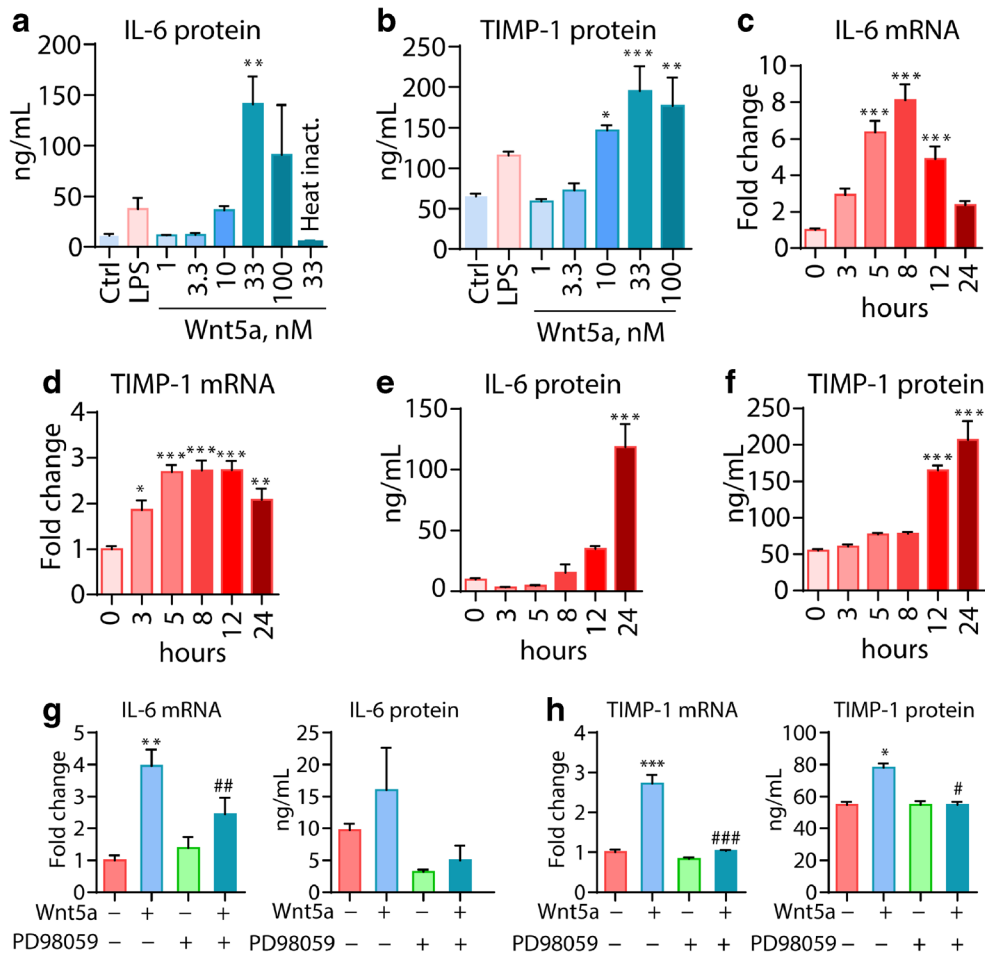
Fig. 6 Wnt5a promotes IL-6 and TIMP-1 release from human cardiac fibroblasts. mRNA is expressed relative to GAPDH. **a** Protein release of IL-6 and **b** TIMP-1 after treatment with increasing doses of Wnt5a for 24 h. **c** Relative mRNA expression of IL-6 and **d** TIMP-1 after treatment with 30 nM Wnt5a at different time points. **e** Protein release of IL-6 and **f** TIMP-1 after treatment with 30 nM Wnt5a at different time points. **g** Relative mRNA expression and protein release of IL-6 and **h** TIMP-1 from CFs treated with recombinant Wnt5a, MEK inhibitor PD98059, or a combination of both for 8 h. Data are mean ± SEM of a representative experiment from three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs control; #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ vs Wnt5a. *IL-6* interleukin-6, *LPS* lipopolysaccharide, *TIMP-1* tissue inhibitor of metalloproteinase 1

could promote myocardial inflammation and fibrosis, and thereby contribute to HF progression. Further mechanistic studies on Wnt5a signaling in HF are needed to elucidate possible therapeutic potential of this novel pathway.

Compliance with ethical standards Human studies conformed to the Declaration of Helsinki and were approved by the South Eastern Regional Committee for Medical and Health Research Ethics. Written informed consent was obtained from all individuals. Animal experiments were approved by the animal research committee and were carried out in accordance with institutional guidelines and conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 2011).

Conflict of interest The authors declare that they have no competing interests.

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