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Growth/differentiation factor 1 alleviates pressure overload-induced cardiac hypertrophy and dysfunction

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ABSTRACT

Pathological cardiac hypertrophy is a major risk factor for developing heart failure, the leading cause of death in the world. Growth/differentiation factor 1 (GDF1), a transforming growth factor-β family member, is a regulator of cell growth and differentiation in both embryonic and adult tissues. Evidence from human and animal studies suggests that GDF1 may play an important role in cardiac physiology and pathology. However, a critical role for GDF1 in cardiac remodelling has not been investigated. Here, we performed gain-of-function and loss-of-function studies using cardiac-specific GDF1 knockout mice and transgenic mice to determine the role of GDF1 in pathological cardiac hypertrophy, which was induced by aortic banding (AB). The extent of cardiac hypertrophy was evaluated by echocardiographic, hemodynamic, pathological, and molecular analyses. Our results demonstrated that cardiac specific GDF1 overexpression in the heart markedly attenuated cardiac hypertrophy, fibrosis, and cardiac dysfunction, whereas loss of GDF1 in cardiomyocytes exaggerated the pathological cardiac hypertrophy and dysfunction in response to pressure overload. Mechanistically, we revealed that the cardioprotective effect of GDF1 on cardiac remodeling was associated with the inhibition of the MEK–ERK1/2 and Smad signaling cascades. Collectively, our data suggest that GDF1 plays a protective role in cardiac remodeling via the negative regulation of the MEK–ERK1/2 and Smad signaling pathways.

1. Introduction

Heart failure is the leading cause of death globally. One of the major risk factors for developing heart failure is pre-existing cardiac remodeling, *i.e.*, cardiac hypertrophy, inflammation, fibrosis, and cardiomyocyte apoptosis [1,20]. Cardiac remodeling may occur with pressure overload (aortic valve stenosis and hypertension), with volume overload (valvular regurgitation), or following cardiac injury, including myocardial infarction, myocarditis, and idiopathic dilated cardiomyopathy [15]. Although the etiologies of these diseases are different, they share molecular, biochemical, and cellular events that collectively change the shape of the myocardium. Distinct remodeling events may initially be beneficial because they are initiated to compensate for cardiac dysfunction, but remodeling ultimately leads to a transition to heart failure [1]. Multiple signaling pathways mediating the development of pathological cardiac remodeling have been identified over the past several decades [5,9,12,15,21,23,31], but the molecular modulators that antagonize the development of cardiac remodeling and the transition to heart failure remain incompletely defined.

Growth/differentiation factor 1 (GDF1) is a transforming growth factor-β family member that was originally isolated from a mouse embryo cDNA library [28]. Two GDF1 transcripts [1.4 kilobases (kb) and 3.0 kb in length] displaying distinct temporal expression patterns were detected in a Northern blot analysis of embryonic mRNA [18]. Furthermore, primary GDF1 protein contains a polybasic proteolytic processing site where it is cleaved to produce a mature protein comprising seven conserved cysteine residues, indicating that GDF1 is post-translationally regulated. Concerning its functional role, GDF1 is a regulator of cell growth and differentiation in both embryonic and adult tissues [2,8]. Recent studies in rodents suggest that GDF1 is involved in establishing left-right asymmetry during early embryogenesis and in neural development during later embryogenesis [3,29]. Mice deficient in GDF1 exhibit a spectrum of defects related to left-right axis formation, including visceral situs inversus, right pulmonary isomerism, and a range of cardiac anomalies [28]. Kaasinen et al. [13] reported that mutations in GDF1 could cause inherited right atrial isomerism, and

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heterozygous loss-of-function mutations in the human GDF1 gene contribute to cardiac defects and vessel remodeling [14]. Furthermore, Wall et al. [30] demonstrated that mature GDF1 activates a Smad2-dependent signaling pathway and is sufficient to reverse the left-right axis. GDF1 overexpression significantly rescues developmental anomalies, such as pericardial edema, circulation failure, and heart malformation, as well as cardiac toxicity caused by morpholinos or arsenite [22]. Together, these findings suggest that GDF1 plays a critical role in cardiac physiology and pathology. However, the role of GDF1 in cardiac remodeling has not been investigated. The current study featured the following objectives: 1) to determine whether GDF1 is altered in dilated cardiomyopathy (DCM) patients and a model of pressure overload-induced cardiac hypertrophy; 2) to determine whether GDF1 expression affects cardiac hypertrophy; and 3) to identify the mechanisms that would be involved in any such effects that are observed.

To this end, we employed knockout (KO) mice with a cardiac-specific deletion of the GDF1 gene and transgenic (TG) mice with cardiac-specific overexpression of GDF1 to determine the role of GDF1 in pathological cardiac remodeling. Our results demonstrate that GDF1-TG mice are resistant to cardiac remodeling via inhibition of MEK–ERK1/2 and Smad signalings, whereas cardiac-specific GDF1-KO mice display the opposite phenotype in response to pressure overload. Our study shows a previously unrecognized therapeutic potential for GDF1 in the treatment of pathological cardiac remodeling and heart failure.

2. Methods

2.1. Antibodies

GDF1 expression in human and mouse samples was determined by Western blotting using a GDF1-specific antibody (R&D Systems, AF858, 1:1000 dilutions). Antibodies to ERK1/2 (#4695, 1:1000 dilution), phospho-ERK1/2$^{Thr202/Thr204}$ (#4370, 1:1000 dilution), MEK1/2 (#9122, 1:1000 dilution), phospho-MEK1/2$^{Ser217/221}$ (#9154, 1:1000 dilution), mTOR (#2983, 1:1000 dilution), phospho-mTOR^{Ser2448} (#2971, 1:1000 dilution), FOXO3A (#2497, 1:1000 dilution), phospho-FOXO3A$^{Ser318/321}$ (#9465, 1:1000 dilution), P38 (#9212, 1:1000 dilution), phospho-P38$^{Thr180/Thr182}$ (#4511, 1:1000 dilution), JNK1/2 (#9258, 1:1000 dilution), phospho-JNK1/2 (#4668, 1:1000 dilution), AKT (#4691, 1:1000 dilution), phospho-AKT^{Ser473} (#4060, 1:1000 dilution), GSK3β (#9315, 1:1000 dilution), phospho-GSK3β (#9322, 1:1000 dilution), FoxO1 (#2880, 1:1000 dilution), phospho-FoxO1^{ser256} (#9461, 1:1000 dilution), and α-actinin (#3134, 1:1000 dilution) were purchased from Cell Signaling Technology (Danvers, MA, USA). The GAPDH (MB001, 1:10,000 dilution) antibody was purchased from Bioworld Technology (Harrogate, UK). Antibodies against atrial natriuretic peptide (ANP, sc20158, 1:200 dilution), lamin B (sc6217, 1:200 dilution), and β-myosin heavy chain MHC (β-MHC, sc53090, 1:200 dilution) were purchased from Santa Cruz Biotechnology (Dallas, TX, USA).

2.2. Study animals

All experiments involving animals were approved by the Animal Care and Use Committee of Renmin Hospital at Wuhan University. Experiments were performed using male mice that were 8–10 weeks of age with body weights of 23.5–27.5 g. These mice were anesthetized with 1.5–2% isoflurane by inhalation or with pentobarbital (30 mg/kg, Sigma) by intraperitoneal injection. The mice were housed with an alternating 12-h light and dark cycle in temperature-controlled rooms and had free access to food and water. The following animals were used.

2.2.1. Cardiac-specific GDF1 conditional knockout mice

Male GDF1-floxed conditional mutation mice (B6.129X1-Gdf1^{tm1Dmus}/Kctt, C57BL/6 background) were ordered from the European Mouse Mutant Archive (EMMA, EM: 02230). To obtain cardiac-specific GDF1 knockout mice, GDF1-floxed mice were crossed with mice that carried the α-MHC-MerCreMer transgene [MEM-Cre-Tg (Myh6-cre/Esr1, Jackson Laboratory, 005650)]. Six-week-old GDF1-Cre mice (with MEM-Cre and the GDF1 genes) were then injected with tamoxifen (80 mg/kg/day, Sigma, T-5648) on 5 consecutive days to induce Cre recombinase expression in these mice. GDF1-Cre mice were identified using a PCR analysis of cardiac genomic DNA with the following primers: primer 1: 5′-ATGCCTTCCTTCAGGTC ACTT-3′, primer 2: 5′-CTCCACATTCGACAGG TCAAA-3′, and primer 3: 5′-GTACTTGG ATCGGTTTGTCTC-3′.

2.2.2. Cardiac-specific GDF1 transgenic mice

Transgenic mice (C57BL/6 background) with cardiac-specific GDF1 expression were generated by subcloning the full-length mouse GDF1 cDNA (Origene, MC202978) downstream of the cardiac α-myosin heavy chain (α-MHC) promoter. The linearized α-MHC-GDF1 plasmid was microinjected into mouse oocytes, which were introduced into pseudopregnant females to obtain the desired transgenic mice. The transgenic mice were confirmed by PCR analyses of tail genomic DNA using the forward PCR primer 5′-ATCTCCCCCATAAGAGTTTGAGTC-3′ and the reverse PCR primer 5′-CCCTGTATCTTCACTCTCAGCC-3′. Four independent lines were obtained for GDF1-Tg mice, and each had the same phenotype.

2.3. Aortic banding

The pressure overload-induced cardiac hypertrophy mouse model via aortic banding (AB) was established as previously described [12,20,23]. After anesthesia and once the absence of reflexes had been established, the left side of the chest of each mouse was opened to identify the thoracic aorta, which was tied against a 26G (for body weights of 25–27.5 g) or 27G (for body weights of 23.5–25 g) needle by a 7–0 silk suture; the needle was then removed, and the thoracic cavity was closed. Finally, adequate constriction of the aorta was determined by Doppler analysis. A similar procedure without aortic constriction was performed in the sham group.

2.4. Treatment of mice with U0126

U0126, an inhibitor of MAPK kinase (MEK) 1/2, was obtained from Cell Signaling Technology (Beverly, MA), dissolved in dimethyl sulfoxide, and administered at a constant volume of 1 ml per 100 g of body weight by intraperitoneal injection every 3 days (1 mg/kg/3 days) [20].

2.5. Echocardiography and hemodynamic measurements

Mice were anesthetized with 1.5–2% isoflurane by inhalation, as described previously [12,20,23,31], and then echocardiography was performed to evaluate left ventricle (LV) function and structure using a Mylab30CV (ESAOTE) machine with a 15-MHz probe. To measure LV end-systolic dimension (LVESD), LV end-diastolic dimension (LVEDD), and LV fractional shortening, M-mode tracings derived from the short axis of the left ventricle at the level of the papillary muscles were recorded; parameters were obtained from at least three beats and averaged. A 1.4-French catheter-tip micromanometer catheter (SPR-839; Millar Instruments) was inserted into the left ventricle via the right carotid artery to obtain invasive hemodynamic measurements. An Aria pressure-volume conductance system coupled with a PowerLab/4SP A/D converter was used to record and store the pressure and dp/dt continuously, which were then displayed on a personal computer.

2.6. Histological analysis

Hearts were arrested with 1 M KCl and fixed in 10% formalin for >24 h. Hearts were paraffin embedded and cut into 5-μm sections.