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Increased kielin/chordin-like protein levels are associated with the severity of heart failure☆

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ABSTRACT

Background: Previous studies demonstrated that the transforming growth factor (TGF) β superfamily, including TGF-βs and bone morphogenetic proteins (BMPs), plays important roles in cardiovascular diseases. The kielin/chordin-like protein (KCP) is a secreted protein that regulates the expression and function of TGF-βs and BMPs. However, the role of KCP during heart failure (HF) remains unknown. The present study aimed to investigate the cardiac expression of KCP in human failing hearts.

Methods: The human failing heart samples from patients with dilated cardiomyopathy (DCM, $n = 12$) and ischemic cardiomyopathy (ICM, $n = 12$) were collected, and normal heart ($n = 8$) samples from unmatched donors were collected as controls. Collagen volume, KCP levels, and mRNA levels of several BMPs in left ventricles (LV) of all hearts were measured.

Results: The KCP levels were significantly higher in human failing hearts than in normal hearts. KCP levels were positively associated with hypertrophy markers, including atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and β-myosin heavy chain (β-MHC). In addition, KCP levels were also positively associated with left ventricular end-diastolic dimension (LVEDD), collagen Iα and collagen IIIα expression but were negatively associated with left ventricular ejection fraction (LVEF). Furthermore, increased TGF-β1, BMP2/4/6/10 and reduced BMP7 levels were observed, and positive correlations between KCP and TGF-β1 and negative correlation between KCP and BMP2/7 were found, but not for BMP4/6/10.

Conclusions: KCP was closely associated with heart failure. The regulation of BMP2/7 and TGF-β1 expression may be the possible mechanisms.

1. Introduction

Chronic heart failure (CHF) is a common clinical disease that is a major cause of death worldwide [1,2] because it may lead to serious clinical complications, including ventricular fibrillation and sudden cardiac death. CHF can be stimulated by various pathological factors, including hypertension, ischemic cardiomyopathy (ICM) and dilated cardiomyopathy (DCM). Although the clinical use of ACEI/ARB and β-blocker improved survival rates in CHF patients, the overall prognosis of CHF remains very poor [2].

Both transformation growth factor (TGF) βs and bone morphogenetic proteins (BMPs) belong to the TGF-β superfamily. It is well known that TGF-βs are involved in several cardiovascular diseases [3]. BMPs are low molecular weight secreted glycoproteins that were initially considered to be useful only for stimulating the formation of bone tissue [4]. In later studies, the versatility of BMPs was found in inflammatory responses, cell proliferation and differentiation, angiogenesis and apoptosis [5]. The best-characterized BMPs in the cardiovascular system are BMP2, BMP4, BMP7 and BMP9 [6]. Previous studies demonstrated that BMP2, BMP4, BMP7 and BMP10 were closely related to the progression of cardiac hypertrophy [7–11]. In addition, BMP3 was also expressed in heart [7], and BMP6 levels were increased in chronic heart failure patients [12]. The regulation by BMPs of cardiac remodeling occurs via the Smad signaling pathway [6].

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Kielin/chordin-like protein (KCP) is a secreted regulator of TGF-β superfamily pathways, down-regulating TGF-β expression but enhancing BMPs levels [13]. Functional investigations of KCP are scarce, primarily focusing on metabolic syndrome, liver and kidney diseases. KCP was shown to protect against unilateral ureteral obstruction (UUO)-induced renal fibrosis via regulating BMP7 expression [14–16]. In addition, KCP attenuated high fat diet-induced obesity and metabolic syndrome [13]. KCP also reduced nonalcoholic fatty liver disease by regulating BMP4 and TGF-β1 [17]. Although a previous study demonstrated that KCP was high expressed in heart [13], the possible function of KCP in cardiovascular disease remains unknown. Based on findings that the TGF-β superfamily plays important roles in cardiovascular diseases and KCP could regulate the TGF-β1 and BMP signaling pathways, we hypothesized that KCP may participate in the development of HF. In the present study, we measured KCP expression in human failing heart samples.

2. Materials and methods

2.1. Collection and procession of human heart samples

This study's protocols were approved by the Medical Ethical Committee of the Renmin Hospital of Wuhan University and the People's Hospital of Guangxi Zhuang Autonomous Region. Normal hearts ($n = 8$) were collected from patients with brain death after a traffic accident or head trauma, and these hearts were originally intended for transplantation but failed to match with suitable recipients. Failing hearts were collected from patients who underwent heart transplantation because of end-stage dilated cardiomyopathy (DCM, $n = 12$) or ischemic cardiomyopathy (ICM, the location of the lesion during these patients was the left ventricle (LV), $n = 12$). The LV tissue in DCM hearts and diseased LV in ICM hearts were collected by cardiac surgeons in Renmin Hospital of Wuhan University, and patients or their families provided informed consent. All procedures related to human samples complied with the principles outlined in the Declaration of Helsinki. Detailed information on the human heart samples is shown in Table 1.

2.2. Histological analysis

The LV tissue was isolated from heart samples and fixed with 4% neutral paraformaldehyde. Then, LV tissue was embedded in paraffin and cut into approximately 4 mm slices. Hematoxylin and eosin (H&E) staining was used for histopathological analysis, and picrosirius red (PSR) and Masson staining was performed to detect collagen expression levels.

2.3. Western blot

LV tissue was lysed and total protein was collected. After measurement with a BCA Protein Assay Kit (Thermo Fisher Scientific, MA, USA), a total of 50 μg protein was added on Laemmli sodium dodecyl sulfate (SDS) polyacrylamide gels for separation. Then, the protein samples were transferred to Immobilon-FL PVDF membranes (Millipore, USA) and were blocked with 5% nonfat milk. After incubation with anti-KCP (Abcam, England) and anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Cell Signaling Technology, USA) antibodies at 4 °C overnight, secondary antibodies were incubated at room temperature for 1 h. The blots were scanned using a two-color infrared imaging system (Odyssey; LI-COR Biosciences, Lincoln, NE, USA).

2.4. Quantitative polymerase chain reaction (RT-qPCR)

TRIZOL Reagent was used to extract the total mRNA of human LV tissue. Then, 2 μg of total mRNA was used to synthesize cDNA using a reverse transcription kit. A LightCycler 480 SYBR Green Master Mix (Roche, Germany) was used to perform PCR amplification. The relative mRNA expression levels of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), β-myosin heavy chain (β-MHC), collagen Iα, collagen IIIα, TGF-β1, BMP2, BMP3, BMP4, BMP6, BMP7 and BMP10 were measured, and mRNA expression was normalized to that of

Table 1
Information of clinical characteristics in normal and failing hearts.

Characteristic	Normal	HF	DCM	ICM
Age (years)	50 ± 11	52 ± 11	54 ± 8	49 ± 14
Gender (M/F)	7/5	15/9	8/4	7/5
Smoking (n, %)	4 (33.3)	10 (41.7)	6 (50)	4 (33.3)
BMI (kg/m^2)	21.6 (19.8–24.4)	20.4 (18.6–21.6)	20.4 (19.0–21.4)	20.0 (18.5–22.2)
HR (bpm)	–	94 ± 16	94 ± 18	95 ± 15
SBP (mmHg)	–	110 (101 − 120)	110 (105–120)	111 (98–120)
DBP (mmHg)	–	72 (66–76)	75 (69–78)	67 (64–72)
EH (n, %)	4 (33.3%)	5 (20.8)	3 (25.0)	2 (16.7)
TC (mmol/l)	4.5 (4.1–4.9)	4.4 (4.3–4.8)	4.4 (4.3–4.9)	4.4 (4.3–4.7)
TG (mmol/l)	1.2 ± 0.3	1.1 ± 0.4	1.0 ± 0.3	1.2 ± 0.5
HDL (mmol/l)	1.1 ± 0.3	1.2 ± 0.4	1.2 ± 0.5	1.2 ± 0.2
LDL (mmol/l)	1.3 ± 0.3	1.4 ± 0.3	1.5 ± 0.2	1.3 ± 0.4
Glu (mmol/l)	5.0 ± 1.0	5.6 ± 0.8*	5.5 ± 0.9*	5.7 ± 0.7*
Albumin (g/l)	42.5 ± 3.6	30.0 ± 3.5*	30.7 ± 3.5*	29.4 ± 3.6*
CREA (μmol/l)	73.3 ± 11.4	109 ± 33*	113 ± 40*	104 ± 26*
LVEF (%)	–	31.0 (29.0–32.8)	31.0 (27.5–32.8)	30.5 (29.0–34.3)
LVEDD (mm)	–	67 ± 5	66 ± 6	68 ± 3
Medications, *n* (%)				
ACEI/ARB	4 (33.3)	21 (87.5)*	11 (91.7)*	10 (83.3)*
β blockers	1 (8.3)	17 (70.8)*	9 (75.0)*	8 (66.7)*
Diuretics	0 (0)	16 (66.7)*	7 (58.3)*	9 (75.0)*
Digitalis	0 (0)	15 (62.5)*	7 (58.3)*	8 (66.7)*
Spironolactone	0 (0)	16 (66.7)*	7 (58.3)*	9 (75.0)*

BMI: body mass index; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; EH: hypertension; TC: total cholesterol; TG: total triglycerides; HDL-C: high-density lipoprotein cholesterol; HDL-C: low-density lipoprotein cholesterol; Glu: fasting glucose; CREA: creatinine; LVEF: left ventricular ejection fraction; LVEDD: left ventricular end-diastolic dimension; DCM: dilated cardiomyopathy; ICM: ischemic cardiomyopathy; ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker.

* $p < .05$ vs. normal.