Type-specific dysregulation of matrix metalloproteinases and their tissue inhibitors in end-stage heart failure patients: relationship between MMP-10 and LV remodelling

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Abstract

Although past studies observed the changes of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) in end-stage heart failure (HF) patients, a consistent and clear pattern of type-specific MMPs and/or TIMPs has yet to be further defined. In this study, proteomic approach of human protein antibody arrays was used to compare MMP and TIMP expression levels of left ventricular (LV) myocardial samples from end-stage HF patients due to dilated cardiomyopathy (DCM) with those from age- and sex- matched non-failing patients. Western blot analysis, immunohistochemistry and ELISA were used for validation of our results. We observed that MMP-10 and -7 abundance increased, accompanied by decreased TIMP-4 in DCM failing hearts ($n = 8$) compared with non-failing hearts ($n = 8$). The results were further validated in a cohort of 34 end-stage HF patients derived from three forms of cardiomyopathies. Cardiac and plasma MMP-10 levels were positively correlated with the LV end-diastolic dimension in this HF cohort. In addition, we observed that insulin-like growth factor-2 promoted MMP-10 production in neonatal rat cardiomyocytes. In conclusion, this study demonstrated a selective up-regulation of MMP-10 and -7 along with a discordant change of TIMP-4, and a positive correlation between MMP-10 levels and the degree of LV dilation in end-stage HF patients. Our findings suggest that type-specific dysregulation of MMPs and TIMPs is associated with LV remodelling in end-stage HF patients, and MMP-10 may act as a novel biomarker for LV remodelling.

Keywords: heart failure • metalloproteinases • left ventricular remodelling

Introduction

Heart failure (HF) is a common cause of morbidity and mortality, and the incidence is increasing [1]. Defects within the myocardial extracellular matrix (ECM) have been identified to directly contribute to left ventricular (LV) remodelling in the HF process [2]. ECM integrity is maintained by a balance between the activity of matrix metalloproteinases (MMPs), a family which comprises more than 25 individual members divided into specific classes based on *in vitro* substrate specificity for various ECM components and tissue inhibitors of metalloproteinases (TIMPs), a family currently com-posed of four members binding and inactivating the various MMPs [3]. In normal physiology, MMPs are involved in embryonic development, wound repair and bone remodelling [4]. In a number of pathologic situations, including during all stages of HF progression, the MMP–TIMP axis plays a crucial role in ECM homeostasis [5]. MMPs are the driving force during myocardial ECM remodelling and selectively increased to mediate collagen degradation leading to LV dilation in animal models of HF and human HF [6, 7]. However, TIMPs are relatively deficient in the failing hearts [8]. Although past studies demonstrated that MMP levels are high and TIMP levels are low in human HF patients [7], a consistent and clear pattern of type-specific MMPs and/or TIMPs has yet to be further defined in end-stage failing human hearts. Accordingly, the main goal of the present study was to clarify the type-specific dysregulation of MMPs and/or TIMPs in end-stage failing human hearts, and to further test whether cardiac and/or plasma MMP-10, one of the MMPs we identified to be up-regulated in end-stage failing human hearts, would be correlated with adverse LV remodelling.

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Materials and methods

Subjects and sample collection

Human LV myocardial samples were obtained from 34 patients diagnosed with end-stage HF due to dilated cardiomyopathy (DCM, $n = 19$), ischemic cardiomyopathy (ICM, $n = 8$) and arrhythmogenic right ventricular cardiomyopathy (ARVC, $n = 7$) undergoing heart transplantation, and eight non-failing control patients. The patients before heart transplantation had a relatively standard therapeutic regimen, including diuretics, digoxin or intravenous inotropes, and ACE inhibitors. Table 1 presents the clinical and hemodynamic characteristics of end-stage HF patients used in this study. All of the patients had no other organ failures or detected diseases. Non-failing control hearts were from donors who died from accident with no history of heart disease. All patients and control patients gave written informed consent for this investigation, which was approved by the Institutional Ethical Review Board of Fuwai Hospital. The investigation also conforms to the principles outlined in the Declaration of Helsinki. According to the new diagnostic criteria [9], all the seven ARVC HF patients we selected belong to 'left dominant' with low LVEF, just like LV dysfunction with low LVEF in 19 DCM and 8 ICM HF patients (Table 1). Therefore, we conformably chose LV myocardial samples from all the HF patients with DCM, ICM and ARVC, so that the results will be comparable among failing hearts due to the three forms of cardiomyopathies to avoid tissue heterogenicity. Samples were collected from LV-free wall of each hearts. Sections of the samples were dissected, snap-frozen in liquid nitrogen, and stored at –70°C until use.

Protein antibody arrays

Total proteins were extracted using standard method [10]. Antibody array-based technology was used to detect multiple proteins present in 8 DCM failing hearts selected from 19 DCM failing hearts (Table 1) and the age- and sex-matched non-failing hearts ($n = 8$) (DCM-1 versus non-failing human hearts (NF)-1, DCM-2 versus NF-2, DCM-5 versus NF-3, DCM-9 versus NF-4, DCM-12 versus NF-5, DCM-15 versus NF-6, DCM-17 versus NF-7, DCM-18 versus NF-8). RayBio® Biotin Label-based Human Antibody Arrays I (507 human protein antibodies were printed on the chips) were purchased from RayBiotech, Inc. (Cat #: AAH-BLM-1–2, Atlanta, GA, USA). The array chips were blocked with blocking buffer, and then equal amounts of biotin-labelled LV myocardial proteins from eight failing and eight matched non-failing hearts were individually added onto glass chips and incubated at 4°C overnight, and bound proteins were then analyzed according to the manufacturer's instructions. The images were captured using an Axon GenePix (Certified GeneTool, Inc., Milpitas, CA, USA) laser scanner, and visualized and analyzed using Genepix pro 6.0 software. A biotinylated protein and internal control will produce positive control signals, which will be used to identify the orientation and help normalize the results from different arrays being compared. Data analysis was performed using the RayBio® Analysis Tool.

Western blot analysis

The relative abundances of MMP-10, MMP-7 and TIMP-4 were examined in individual LV myocardial protein extracts using standard immunoblotting procedures as described previously [10]. The primary antibodies used included polyclonal rabbit anti-human antibodies for MMP-10 (1:1000, Cat. # GTX24045, GeneTex, Inc., San Antonio, TX, USA), MMP-7 (1:500, Cat. # 10374–2-AP, Protein Tech Group, Inc., Chicago, IL, USA) and TIMP-4 (1:250, Cat. # ab2170, Abcam Inc., Cambridge, MA, USA), as well as monoclonal mouse anti-human antibody for glyceraldehyde-3-phosphate dehydrogenase (GADPH) (1:2000, Cat. # ab9484, Abcam, Inc.)

Immunohistochemical analyses

LV myocardial samples was harvested and fixed in 10% neutral buffered formalin. Dehydration was accomplished through alcohol and xylene gradients, followed by embedding in paraffin. Sections (5 μm) were fixed for 10 min. in 4% paraformaldehyde, permeabilized with 0.2% Triton X-100 for 5 min. and blocked in 5% BSA. They were then incubated with primary antibodies for 1 hr at room temperature and washed in PBS buffer for 10 min., followed by incubation with IgG-peroxidase conjugated secondary antibody (Sigma, St. Louis, MO, USA) for 1 hr at room temperature, washed in PBS buffer for 10 min. and incubated with 0.5 mg/ml diaminobenzidine tetrahydrochloride 2-hydrate plus 0.05% H_2O_2 for 5 min. All the slides were stained with hematoxylin, dehydrated, mounted, and viewed by light microscope. The primary antibodies used included rabbit anti-human antibodies for MMP-10 (1:50, Cat. # GTX24045, GeneTex, Inc.), MMP-7 (1:50, Cat. # 10374–2-AP, Protein Tech Group, Inc.) and TIMP-4 (1:50, Cat. # ab2170, Abcam, Cambridge, UK), as well as mouse anti-human GAPDH antibody (Cat. # ab9484, Abcam MA, USA).

Cardiac and plasma MMP-10 measurement by ELISA

Total proteins were extracted individually using standard method [10]. Blood samples were collected into EDTA-containing Vacuette tubes (Greiner Bio-One) and centrifuged at $3000 \times g$ (15 min., +4°C), and subsequently stored at –70°C until batch analysis was performed. Cardiac and plasma MMP-10 was assessed using ELISA. The commercial human MMP-10 ELISA Kit (Cat. # LH872, RapidBio Lab, Calabasas, California, USA) was used in this study according to the manufacturer's protocols. The resultant reaction was read at a wavelength of 450 nm (Labsystems Multiskan MCC/340, Helsinki, Finland). All samples were analyzed in duplicate and averaged.

Cell culture and treatment

Primary neonatal rat ventricular myocytes were isolated and cultured as described previously [11]. After the cells started beating 48 hrs after culture, they were treated with or without insulin-like growth factor-2 (IGF-2) (10^{-9} to 10^{-6} M; Sigma Chemical Co., St Louis, MO, USA). After further incubation for 24 hrs, the cells were harvested for ELISA using rat MMP-10 ELISA kit (Cat. # E0098r, USCN Life Science & Technology Company, Missouri, TX, USA) and for Western blot and immunocytochemical analyses using rabbit anti-rat MMP-10 antibody (1:50 and 1:10, Cat. # LS-B1230, Lifespan Biosciences, Inc., Seattle, WA, USA) according to the foresaid methods. The procedure using rats for primary neonatal cardiomyocyte culture was approved by the Animal Laboratory Use and Care Committee at Fuwai Hospital. Studies also conformed to the Guide for the Care and Use of Laboratory Animals (NIH Publication 85–23, revised 1996).

Table 1 Clinical and hemodynamic characteristics of end-stage HF patients

Group	Sex	Age (years)	NYHA class	CI	PAP	PVR	CVP	PAWP	LVEDD	LVEF
DCM-1	M	29	IV	2.05	60/39	286	11	35	54	20
DCM-2	M	40	IV	1.73	64/33	294	12	31	77	20
DCM-3	M	55	IV	1.94	59/17	239	2	21	82	29
DCM-4	M	63	III	2.60	35/20	85	11	20	72	25
DCM-5	M	46	IV	3.38	75/29	497	11	22	80	33
DCM-6	M	52	IV	3.03	59/39	132	8	40	81	20
DCM-7	M	63	IV	1.44	79/32	681	3	23	88	27
DCM-8	M	54	IV	1.21	60/32	117	9	32	80	20
DCM-9	M	30	IV	1.61	67/36	385	20	33	62	33
DCM-10	M	61	III	1.70	76/37	393	8	37	76	30
DCM-11	M	42	III	1.89	60/25	338	10	22	83	23
DCM-12	M	42	III	1.36	74/43	415	16	38	77	27
DCM-13	M	45	IV	2.09	64/35	355	9	27	69	24
DCM-14	M	18	IV	1.46	67/44	810	9	28	68	24
DCM-15	M	39	III	1.88	80/47	662	19	32	85	20
DCM-16	F	18	III	2.88	28//6	91	−3	8	71	30
DCM-17	F	49	III	2.22	47/24	364	5	16	70	22
DCM-18	F	59	IV	1.85	61/32	358	7	27	67	22
DCM-19	F	13	IV	1.95	22/15	123	18	13	42	15
ICM-1	M	51	IV	2.42	46/18	274	4	24	78	25
ICM-2	M	50	IV	3.36	45/20	394	−4	3	72	40
ICM-3	M	39	IV	1.96	48/28	306	3	20	90	25
ICM-4	M	45	IV	1.85	51/25	98	9	29	74	32
ICM-5	M	48	IV	1.84	84/36	836	9	13	66	25
ICM-6	M	63	IV	2.04	34/17	404	0	4	74	39
ICM-7	M	46	IV	2.07	53/26	332	6	18	74	11
ICM-8	M	42	III	2.08	55/24	227	4	24	93	15
ARVC-1	M	54	III	2.76	30/13	125	9	10	65	24
ARVC-2	M	52	IV	1.95	53/23	370	4	16	73	24
ARVC-3	M	50	III	2.33	76/40	463	15	25	83	37
ARVC-4	F	16	IV	2.5	24/15	64	15	15	61	15
ARVC-5	F	41	III	1.87	15/5	183	−1	1	60	22
ARVC-6	F	51	III	1.59	31/13	271	16	9	62	24
ARVC-7	F	50	III	1.81	44/33	418	6	30	64	41
NF-1	M	30								
NF-2	M	38								
NF-3	M	46								
NF-4	M	31								
NF-5	M	42								
NF-6	M	37								
NF-7	F	51								
NF-8	F	58								

DCM: dilated cardiomyopathy; ICM: ischemic cardiomyopathy; ARVC: arrythmogenic right ventricle cardiomyopathy; NF: non-failing control; NYHA class: New York Heart Association functional class; CI: cardiac index (L/min.m^2); PAP: pulmonary artery pressure (mmHg); PVR: pulmonary vascular resistance (dyn.S. cm^{-5}); CVP: central vein pressure (mmHg); PAWP: pulmonary artery wedge pressure (mmHg); LVEDD: left ventricular end-diastolic dimension (mm); LVEF: left ventricular ejection fraction (%).

Table 2 Proteins with increased expression as defined by $P < 0.01$ in DCM failing hearts compared with non-failing hearts

NCBI	Protein name	DCM failing hearts ($n = 8$)	Non-failing hearts ($n = 8$)
CAA57890	Activin C	2.53 ± 1.42	0.33 ± 0.07
P29965	CD40 Ligand	1.29 ± 0.12	0.31 ± 0.09
AAQ 67702	E-Selectin	2.57 ± 1.33	0.45 ± 0.13
AAQ88824	FAM3B	2.03 ± 1.31	0.20 ± 0.06
BAA07320	Fas Ligand	1.79 ± 0.65	0.25 ± 0.05
BAA99415	FGF-21	1.66 ± 0.57	0.29 ± 0.06
NP_002297	Galectin-3	1.75 ± 0.77	0.38 ± 0.06
AAH05278	Glucagon	4.55 ± 1.92	0.52 ± 0.07
NP_008862	Glut3	2.15 ± 1.06	0.26 ± 0.05
NP_990758	IFN-α	6.38 ± 1.74	0.18 ± 0.06
NP_001007140	IGF-2R	3.68 ± 1.34	0.21 ± 0.05
AAM77569	IL-17RC	1.94 ± 0.63	0.37 ± 0.06
P22064	Latent TGF	1.32 ± 0.07	0.31 ± 0.06
NP_002414	MMP-7	2.98 ± 0.99	0.77 ± 0.05
NP_002416	MMP-10	11.16 ± 2.60	1.61 ± 0.07
AAQ67703	P-selectin	3.47 ± 0.79	0.73 ± 0.02
CAA10169	RAGE	3.39 ± 1.04	0.46 ± 0.08
NP_003003	sFRP-1	1.42 ± 0.28	0.30 ± 0.05

Table 3 Proteins with decreased expression as defined by $P < 0.01$ in DCM failing hearts compared with non-failing hearts

NCBI	Protein name	DCM failing hearts ($n = 8$)	Non-failing hearts ($n = 8$)
CAG46758	Csk	0.13 ± 0.01	1.52 ± 0.37
AAH96229	GDF9	2.89 ± 1.34	13.09 ± 4.01
NP_002181	GRO-a	2.25 ± 0.94	10.05 ± 3.94
AAA59172	Insulin	0.73 ± 0.27	2.88 ± 0.16
NP_000202	MAC-1	0.21 ± 0.08	2.08 ± 0.15
NP_001515	Orexin B	0.35 ± 0.09	2.19 ± 0.45
NP_937827	Osteocrin	0.05 ± 0.01	1.12 ± 0.02
P16234	PDGF Rα	0.75 ± 0.33	7.55 ± 1.23
NP_002955	SI00 A8/A9	1.03 ± 0.92	7.73 ± 0.73
AAH07022	SAA	0.10 ± 0.01	1.87 ± 0.12
NP_001001420	Smad 5	0.32 ± 0.07	1.83 ± 0.51
EAW62931	Smad 7	0.22 ± 0.05	1.59 ± 0.77
NP_055234	Soggy-1	0.20 ± 0.06	1.28 ± 0.13
AAI30562	TECK / CCL25	0.23 ± 0.04	1.83 ± 0.12
PI0646	TFPI	0.06 ± 0.01	1.26 ± 0.06
NP_003247	TIMP-4	0.12 ± 0.02	1.09 ± 0.02
NP_005109	TL1A / TNFSF15	0.12 ± 0.01	0.83 ± 0.05
AAQ89953	XEDAR	0.05 ± 0.01	1.34 ± 0.12

Statistical analysis

All data are presented as mean ± standard deviation. SPSS software (SPSS Inc., Chicago, IL, USA) for windows 11.0 was used for all statistical analyses. Differences between two groups were compared using an ANOVA. A values of $P < 0.05$ was considered statistically significant. Simple linear regression analysis was performed to examine the correlation between two variables.

Results

Cardiac MMP and TIMP profiles

LV myocardial protein samples from eight DCM failing hearts and eight age- and sex-matched non-failing hearts were individually hybridized with RayBio® Biotin Label-based Human Antibody Arrays I. The expression levels of 507 human proteins can be simultaneously detected, including cytokines, chemokines, growth factors, angiogenic factors, proteases and protease inhibitors, and soluble receptors. Compared to non-failing hearts,

we found 36 proteins to be commonly and consistently deregulated across the eight DCM failing heart samples (all $P < 0.01$). Among them 18 proteins were up-regulated (Table 2) and 18 proteins were down-regulated (Table 3) in DCM failing hearts. In this study, our interest is focused on MMPs and TIMPs. The antibody arrays we used can detect 17 species of main MMPs and all the 4 species of TIMPs simultaneously. In our DCM failing hearts, cardiac MMP and TIMP profiles presented a significant up-regulation of both MMP-10 and -7 and a significant down-regulation of TIMP-4 compared with non-failing hearts (all $P < 0.01$) (Fig. 1), whereas the signal intensity of the remaining MMP and TIMP species did not change significantly.

Validation

To validate the results from protein antibody arrays, we performed Western blot analysis not only in DCM failing human hearts ($n = 19$, including the 8 DCM failing human hearts for initial hybridization with antibody arrays), but also in ICM ($n = 8$) and ARVC failing human hearts ($n = 7$). The target protein levels related to the internal standard protein GAPDH were calculated as the relative abundance and are illustrated in Figure 2. Western
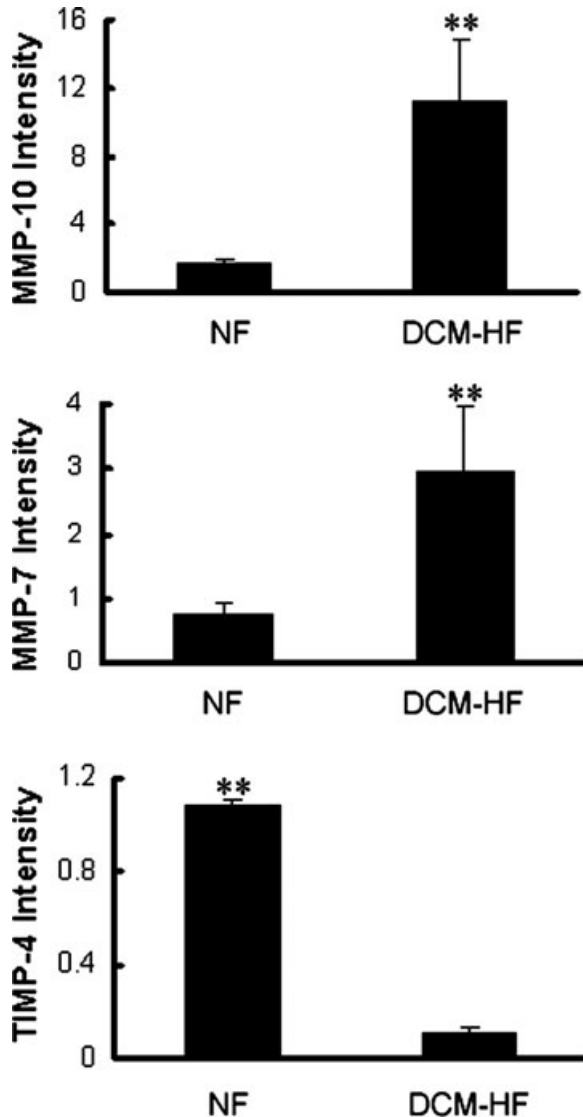
Fig. 1 The signal intensity of cardiac MMP-10 and -7 increased and that of cardiac TIMP-4 decreased in DCM HF patients compared with those in non-failing patients (**$P < 0.01$). LV myocardial samples used in this study for hybridization with protein antibody array chip were from eight DCM HF patients (DCM-HF) and eight age- and sex-matched non-failing subjects (NF).

blot analysis confirmed the increase in cardiac MMP-10 and -7 abundance and the decrease in cardiac TIMP-4 abundance in failing human hearts due to DCM, ICM and ARVC when compared with non-failing human hearts ($n = 8$) ($P < 0.05$ or $P < 0.01$). The results from Western blot analysis are consistent with those from protein antibody arrays.

We further examined the expression of cardiac MMP10, MMP-7 and TIMP-4 in failing and non-failing hearts by immunohistochemistry. Consistent with the results from protein antibody arrays and Western blot analysis, strongly immunoreactive

MMP-10 and -7, and weekly immunoreactive TIMP-4 were observed in failing human hearts due to DCM, ICM and ARVC in contrast to control specimens from non-failing hearts (Fig. 3). The diffuse staining pattern of these proteins in myocardium might reflect the fact that they are secreted.

Cardiac and plasma MMP-10 measurement by ELISA

Cardiac and plasma MMP-10 levels were increased in HF patients with DCM ($n = 19$), ICM ($n = 8$) and ARVC ($n = 7$) when compared with non-failing patients (Fig. 4A and B, $P < 0.05$ or $P < 0.01$). Linear regression analysis showed that cardiac MMP-10 abundance was positively correlated to plasma MMP-10 levels in HF patients derived from the three forms of cardiomyopathies (Fig. 4C, $n = 34$, $r = 0.89$, $P < 0.001$)

Correlation between MMP-10 and LV dilation

To determine which parameters in Table 1 correlated with MMP-10, linear regression analyses were performed using MMP-10 as the independent variable. A positive linear correlation was observed with respect to both cardiac and plasma MMP-10 levels and LV end-diastolic dimension (LVEDD), which indicates LV remodelling and dilation, in the HF patients derived from the three forms of cardiomyopathies (Fig. 5A and B). No significant correlation was observed between MMP-10 and each of the other parameters listed in Table 1.

Regulation of IGF-2 on MMP-10 production

ELISA revealed that IGF-2 induced a significant increase of cellular MMP-10 after incubation with IGF-2 (10^{-6} M) for 24 hrs in primary neonatal rat cardiomyocytes (Fig. 6A). Western blot (Fig. 6B) and immunocytochemical analysis (Fig. 6C) further demonstrated that IGF-2 promoted the immunoreactive MMP-10 production after incubation with IGF-2 (10^{-6} M) for 24 hrs in primary neonatal rat cardiomyocytes.

Discussion

Although the causes of HF are diverse, a common outcome in the progression of this disease process is ECM remodelling, resulting in cardiac dilation and loss of contractility. MMPs are the driving force during ECM remodelling and are selectively increased to mediate ECM degradation leading to LV dilation in animal models of HF and human HF [5–7]. For example, using specific antisera directed against MMPs, increased levels of MMP-2, -3 and -9 in
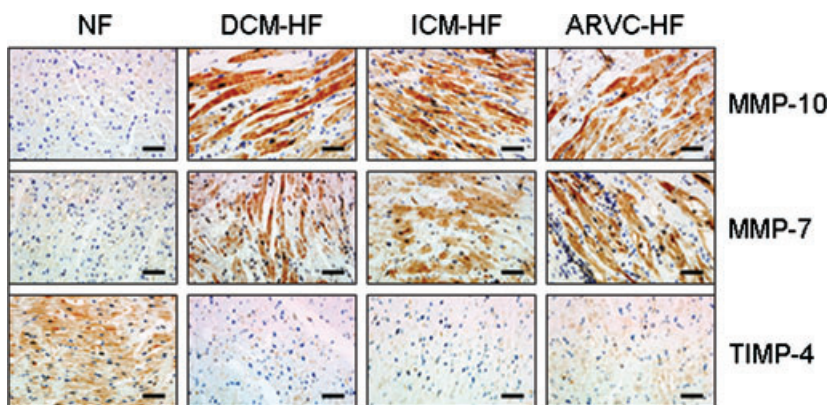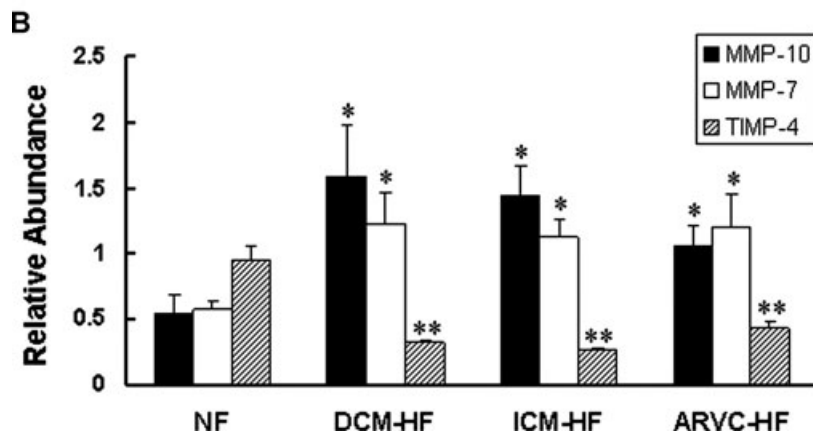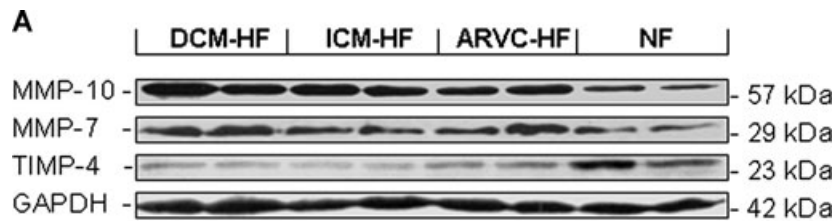
Fig. 2 (**A**) Representative Western blot analysis of cardiac MMP-10, MMP-7 and TIMP-4 expression. MMP and TIMP types are shown at left and molecular weights at right. (**B**) Densitometric quantification of cardiac MMP-10, MMP-7 and TIMP-4 protein expression. The relative abundance represents quantitative results from 19 DCM failing human hearts (DCM-HF), 8 ICM failing human hearts (ICM-HF), 7 ARVC failing human hearts (ARVC-HF), and 8 non-failing human hearts (NF). Densitometric analyses of the blots showed a robust increase in MMP-10 and -7 protein abundance and decrease in TIMP-4 protein abundance in failing hearts due to the three forms of cardiomyopathies compared with non-failing hearts (*$P < 0.05$ or **$P < 0.01$).

Fig. 3 Representative immunohistochemical analysis of cardiac MMP-10, MMP-7 and TIMP-4 expression in failing and non-failing hearts. Strong immunostaining for cardiac MMP-10 and -7, and weak immunostaining for cardiac TIMP-4 were observed in failing human hearts due to DCM (DCM-HF), ICM (ICM-HF) and ARVC (ARVC-HF) in contrast to non-failing hearts (NF). Scale bar = 50 μm.

LV myocardium from end-stage HF patients were observed [6]. MMPs that are expressed at very low levels in normal myocardium, such as MMP-13 and MMP-14, were substantially up-regulated in HF [5]. These above observations indicate that MMP species are not uniformly increased in HF patients, suggesting that a specific portfolio of MMPs is expressed in the failing myocardium. A loss of TIMP-mediated inhibitory control on MMPs has been reported in the failing human hearts. For example, TIMP-1 and -3 are significantly down-regulated in both DCM and ICM, whereas TIMP-4 is down-regulated in ICM [12]. This disparity between MMP and TIMP levels favours a persistent MMP activation state within the myocardium and likely contributes to the ECM remodelling process in HF [5]. The molecular basis for a selective portfolio of MMPs to be increased within the failing human myocardium is likely due to the type, degree and duration of the specific extracellular stimuli that are present [5]. Although past studies demonstrated that selective MMP levels are high and selective TIMP levels are low in HF patients, a consistent and clear pattern of type-specific MMPs and/or TIMPs has yet to be further defined in end-stage HF patients.

An important cause of HF is DCM [7]. Thus, in the present study, we used DCM failing LV myocardial samples and age- and sex-matched non-failing LV myocardial samples to screen and identify type-specific dysregulation of MMPs and/or TIMPs that may play a role in ECM remodelling and LV dilation in end-stage HF patients by proteomic approach of human protein antibody
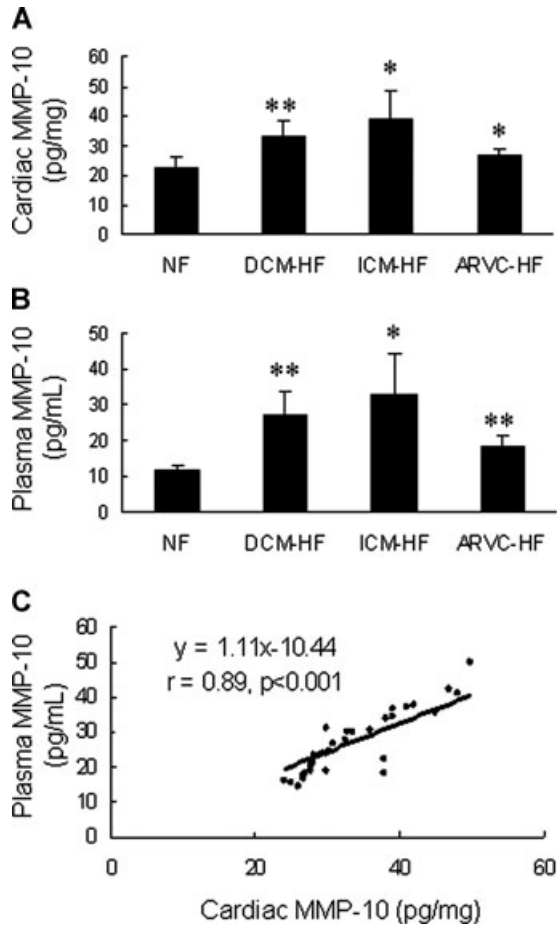
Fig. 4 MMP-10 measurements in HF patients and non-failing subjects (NF). (**A**) and (**B**) Cardiac MMP-10 abundance and plasma MMP-10 levels were increased in HF patients derived from DCM (DCM-HF, $n = 19$), ICM (ICM-HF, $n = 8$) and ARVC (ARVC-HF, $n = 7$) compared with NF (NF, $n = 8$) ($*P < 0.05$ or $**P < 0.01$). (**C**) Linear regression analysis showed cardiac MMP-10 abundance was correlated positively with plasma MMP-10 levels in HF patients derived from the three forms of cardiomyopathies ($n = 34$, $r = 0.89$, $P < 0.001$).

Fig. 5 Correlation of MMP-10 with LV dilation in HF patients derived from three forms of cardiomyopathies ($n = 34$). (**A**) Higher levels of Cardiac MMP-10 were correlated with higher values of LVEDD ($r = 0.80$, $P < 0.001$). (**B**) Higher levels of plasma MMP-10 were correlated with higher values of LVEDD ($r = 0.77$, $P < 0.001$). LVEDD: left ventricular end-diastolic dimension (mm).

arrays, which can detect 17 species of main MMPs and all 4 species of TIMPs. We demonstrated a significant up-regulation of MMP-10 and -7 along with a down-regulation of TIMP-4 in DCM end-stage failing hearts compared with non-failing hearts, which were further validated by Western blot and immunochemistry not only in the failing hearts due to DCM, but also in the failing hearts due to ICM and ARVC. In view of that past studies have reported that both increased MMP-7 and decreased TIMP-4 are in relation to HF and myocardial ECM remodelling [12, 13], our particular interest is mainly focused on the relation between MMP-10 and myocardial ECM remodelling. MMP-10 degrades multiple components of the ECM, such as proteoglycan, laminin, fibronectin and collagen III and IV [14]. Past studies demonstrated that the up-regulation of MMP-10 led to the abnormalities in vascular integrity
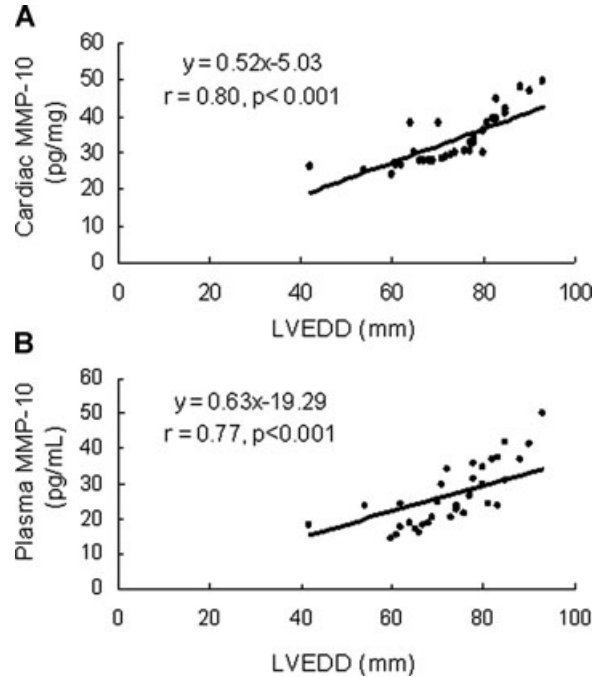
[15]. MMP-10 acted as a potential biomarker and a relevant molecule in atherosclerotic vascular remodelling [16]. MMP-10 has also been shown to be associated with aortic aneurysm, characterized by destructive remodelling of the vascular ECM and rupture of the vessel wall, and are likely to contribute to disease pathogenesis [17]. Although MMP-10 has been related to vascular pathophysiology, there are few reports investigating the relationship between MMP-10 and myocardial ECM remodelling in end-stage HF patients.

We demonstrated that up-regulation of cardiac and plasma MMP-10 is the common change in the cohort of our end-stage HF patients. The correlation of MMP-10 with myocardial ECM remodelling is not clear yet. Our unique finding was that cardiac and plasma MMP-10 were positively correlated with the LV dilation in our HF patients. Myocardial sampling is not readily amenable for clinical application for diagnostic/prognostic purposes or for the evaluation of a specific therapeutic intervention. Therefore, plasma levels of certain MMPs, such as MMP-2 and -3, were determined in the past reports [1, 18]. Our results suggest that plasma MMP-10 levels might be considered as a novel biomarker in the development of diagnostic criteria and the design of novel therapeutic management strategies for myocardial ECM remodelling in end-stage HF patients. As with any blood-derived biomarker, MMP-10 plasma levels only serve as surrogate markers of a localized
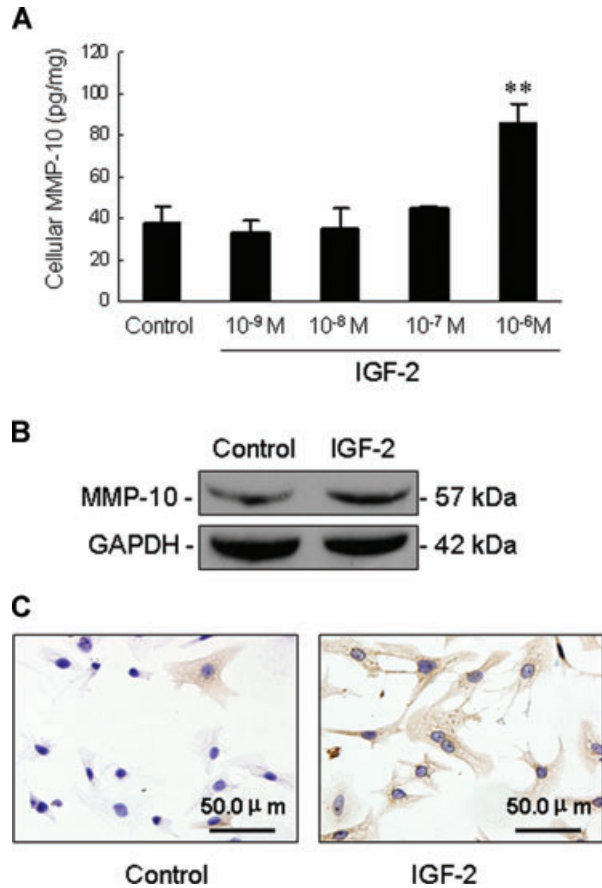
Fig. 6 Regulation of IGF-2 on MMP-10 production in neonatal rat cardiomyocytes. (**A**) ELISA revealed that IGF-2 induced a significant increase of cellular MMP-10 after incubation with IGF-2 (10^{-6} M) for 24 hrs compared with the control (**$P < 0.01$). The data for *in vitro* study were obtained from three independent experiments. (**B**) Representative Western blot indicated that IGF-2 promoted the immunoreactive MMP-10 production in cardiomyocytes after incubation with IGF-2 (10^{-6} M) for 24 hrs compared with the control. The internal standard GAPDH was used to normalize for equal protein loading. (**C**) Representative immunocytochemical staining showed that few immunoreactive MMP-10 cells were observed in neonatal rat cardiomyocytes cultured normally (left), but more immunoreactive MMP-10 cells were seen when cardiomyocytes were incubated with IGF-2 (10^{-6} M) for 24 hrs (right). Scale bar = 50 μm.

process that is occurring within the remodelling myocardium. Thus this approach is dependent on adequate spillover of MMP-10 from remodelling myocardium. Thus MMP-10 plasma levels may not be reflective of myocardial concentrations. In our HF patients, MMP-10 plasma levels were correlated positively with cardiac MMP-10 abundance, suggesting that MMP-10 plasma levels might reflect spillover from the remodelling myocardium and the myocardium might be an important source of circulating MMP-10. Further studies that use a large, prospective, and serial study design are required in order to elucidate whether systemic

MMP-10 levels may represent a new biomarker for myocardial ECM remodelling, and to better define the role for MMP-10 in the status of end-stage HF.

The myocardial ECM remodelling is regulated by MMPs, which in turn are regulated by cytokines. Increased levels of specific cytokines with the development of HF may modulate MMP species abundance [6, 19], and experimental studies have demonstrated that the production of MMPs is increased in cardiac as well as other cells after stimulation by the cytokines [20, 21]. Past studies demonstrated that elevated levels of IGF-2, acting as one of important cytokines, involved in adverse cardiac remodelling in a rat model of hypertension [22] and resulted in significant increase in the MMP-9 in H9c2 cardiomyoblast cells [23]. IGF-2 suppresses protein degradation and supports protein synthesis and the uptake of amino acids [24]. IGF-2 is also a mitogen for different cell types in pathological myocardium [25]. It appears possible that IGF-2 exerts a protective role in myocardium by aiding the survival of myocytes after damage. In our study, we observed a significant increase of cardiac IGF-2 receptor (IGF-2R) coupled with the increase of cardiac MMP-10 in the failing hearts when compared with non-failing hearts using the same protein antibody array approach (Table 2). Thus, this study tested the hypothesis that IGF-2 would involved in the regulation of cardiac MMP-10 production *in vitro*. To our knowledge, we are the first to demonstrate that IGF-2 stimulated the production of MMP-10 in cultured neonatal rat cardiomyocytes. Although IGF-2 may be only one of the multiple signalling pathways that are operative in end-stage HF, which likely contribute to alterations in cardiac levels of MMP-10, our results suggest a cause-and-effect relationship between IGF-2 stimulation and MMP-10 production.

Limitations of this study

First, samples were obtained from patients in end-stage HF treated with medications, for example, ACE inhibitor. It is likely that specific pharmacologic interventions may influence cardiac MMP and TIMP abundance. Thus future studies that use animal models of LV dilation and failure may provide insight into the mechanism(s) that regulate MMP-10, -7 and TIMP-4 expression. Second, in this study, we did not establish the cellular localization of MMP-10. All cell types found in the heart, either under basal conditions (cardiomyocytes, fibroblasts, endothelial cells) or in response to a pathological stimulus (neutrophils and macrophages) express one or more types of MMP species [26]. The cell types responsible for MMP-10 production remain unknown. Based on our results that cultured cardiomyoctes expressed MMP-10, cardiomyocyte is at least one of the important cellular localization of MMP-10. Third, cardiac MMP and TIMP abundance measured in this study may not reflect their true 'activity'. MMP and TIMP activity may be better assessed by zymographic methods, although several studies have reported that increased MMP abundance reflects increased MMP zymographic activity in cardiac samples from HF patients

with cardiomyopathic disease [2, 6]. Last, although we observed close correlation between elevated MMP-10 and LV dilation in the failing hearts, we cannot directly establish a cause and effect relationship between MMP-10 and adverse LV remodelling. However, results from the targeted gene studies in animals [27], the population-based studies in human beings [28], and the broad-spectrum MMP inhibition studies [29], provided mechanistic and observational support for the cause-effect relationship between MMP induction and adverse LV remodelling.

Conclusions

Our new and unique findings were 3-fold. First, a selectively significant up-regulation of MMP-10 and -7 along with a discordant change of TIMP-4 was observed in the end-stage failing human hearts. Second, increased MMP-10 was closely correlated with LV dilation in end-stage HF patients. Third, MMP-10 was induced in response to IGF-2 activation in cultured cardiomyocytes. Our findings suggest that type-specific dysregulation of MMPs and TIMPs is associated with LV remodelling in end-stage HF patients, and MMP-10 may act as a novel biomarker for LV remodelling.

Acknowledgements

Conflict of interest

None declared.

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