

Altered Expression of Disintegrin Metalloproteinases and Their Inhibitor in Human Dilated Cardiomyopathy

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Background—Disintegrin metalloproteinases (ADAMs) may contribute to structural cardiac remodeling by altering cell-surface matrix receptors (integrins) and activating potent biomolecules. We compared expression of ADAMs, their endogenous inhibitor tissue inhibitor of metalloproteinases (TIMP)-3, and integrins in human heart tissue with varied patterns of structural remodeling.

Methods and Results—Myocardium was obtained from patients with dilated cardiomyopathy (n=20), hypertrophic obstructive cardiomyopathy (n=5), and nonfailing donor hearts (n=7). Paired samples (n=10) were obtained before left ventricular assist device insertion and at transplantation. The expressions of ADAM10, ADAM12, ADAM15, and ADAM17, TIMP-3, and integrin receptors β 1D and β 3 were determined by quantitative immunoblotting. Integrin shedding was assessed by the ratio of integrin cleavage products to intact protein abundance. Confocal microscopy was performed. Dilated cardiomyopathy was characterized by increased ADAM10 and ADAM15 expression and reduced TIMP-3 expression. The integrin β 1D cleavage ratio was elevated, indicating receptor shedding. ADAM10 and ADAM15 expressions correlated with the cleavage ratio. ADAM10 colocalized with integrin β 1D by confocal microscopy. ADAM10 expression correlated with clinical indices of chamber dilatation and systolic dysfunction. Hemodynamic unloading reduced ADAM10 and ADAM12 expressions and increased integrin β 1D expression. ADAM12 and integrin β 1D expressions were increased in HOCM. ADAM17 was increased in both dilated cardiomyopathy and hypertrophic obstructive cardiomyopathy.

Conclusions—Disintegrin metalloproteinases are differentially expressed in human myocardium, reflecting the underlying pattern of structural remodeling. ADAM10 and ADAM15 may contribute to cardiac dilatation by reducing cell-matrix interactions via integrin shedding. Targeting disintegrin metalloproteinases, perhaps by restoring deficient TIMP-3 levels with gene or cell-based therapies, may prevent progressive chamber dilatation in human dilated cardiomyopathy. (*Circulation*. 2006;113:238-245.)

Key Words: cardiomyopathy ■ surgery ■ transplantation ■ metalloproteinases ■ remodeling

The clinical progression of congestive heart failure is largely determined by a dynamic and seemingly relentless process of cardiac remodeling.¹ Structural cardiac remodeling, such as ventricular dilatation with increased sphericity, involves cellular and extracellular matrix disruption with altered cell-cell and cell-matrix contacts. Matrix metalloproteinases (MMPs) are matrix-degrading proteases that are activated in the failing heart and contribute to structural cardiac remodeling.² The extended metalloproteinase family includes the disintegrin metalloproteinases. These unique MMPs are also known as ADAM enzymes (A Disintegrin And Metalloproteinase), capable of activating potent biomol-

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ecules such as inflammatory cytokines and directly altering cell-surface matrix receptors (integrins) through their disintegrin domain. Excessive MMP activity can profoundly influence tissue architecture, and the multifunctional roles of ADAMs may provide insights into novel underlying mechanisms. Despite the promise of disintegrin metalloproteinases in understanding the coordinated control of structural cardiac remodeling, the pattern of ADAM expression and the role of specific ADAM enzymes in human cardiac remodeling and the progression of heart failure are unknown.

Received June 24, 2005; revision received October 2, 2005; accepted October 28, 2005.

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Circulation is available at <http://www.circulationaha.org>

DOI: 10.1161/CIRCULATIONAHA.105.571414

We surmised that disintegrin metalloproteinases are differentially expressed in the failing human heart in a profile that parallels the underlying pattern of structural cardiac remodeling. We hypothesized that increased activity of specific ADAMs contributes to structural cardiac remodeling by altering cell-matrix and cell-cell contact through shedding of integrin receptors. We previously documented a role for tissue inhibitor of metalloproteinases (TIMP)-3 deficiency in structural cardiac remodeling.^{3,4} Accordingly, we selected a profile of ADAM enzymes inhibited by TIMP-3⁵⁻⁸ and those previously implicated in structural cardiac remodeling.⁹ To this end, we compared the differential myocardial expression of ADAM10, ADAM12, ADAM15, and ADAM17, their endogenous inhibitor TIMP-3, and selected integrin receptors in patients with dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy with preserved systolic function, and "normal" non-failing human myocardium. In addition, DCM samples were compared before and after left ventricular assist device (LVAD) therapy to understand the role of these unique proteases in reverse structural remodeling.

Methods

Human Cardiac Tissue Sample Collection

Myocardial samples of the left ventricle (LV) were collected during cardiac surgery at the time of cardiac transplantation or insertion of a mechanical assist device. All procedures involving human tissue use were approved by the institutional review boards of the Toronto General Hospital, University of Toronto, and the Cleveland Clinic Foundation. Consent was obtained from patients before tissue harvest. Transmural samples were collected whenever possible, avoiding obvious areas of scar or infarction. Samples were immediately snap-frozen in liquid nitrogen and stored at -80°C until use.

LV myocardial samples were obtained from 32 patients at the time of cardiac surgery (25 from the Cleveland Clinic Foundation and 7 from Toronto General Hospital, University of Toronto). Samples were collected from patients with DCM ($n=20$) at the time of cardiac transplantation. Paired serial samples were obtained in 10 of these DCM patients at the time of implantation of an LVAD and again when the heart was excised for cardiac transplantation ($n=20$ samples). Additional patients samples included those with hypertrophic obstructive cardiomyopathy (HOCM) ($n=5$) undergoing surgical resection of LV outflow tract obstruction and patients with structurally normal nonfailing hearts collected at the time of organ procurement for transplantation (NF group) ($n=7$).

Immunoblotting and Quantification of Protein Expression

The relative abundance of ADAM10, ADAM12, ADAM15, ADAM17, TIMP-3, and integrin $\beta 1\text{D}$ and $\beta 3$ was examined in LV myocardial extracts with the use of standard immunoblotting procedures as described previously.³ In brief, protein extracts were prepared from human heart tissue by extraction with Tris-based lysis buffer: 0.05 mol/L Tris, pH 7.6, 0.5% Triton X-100, 0.3 mol/L NaCl, 5 mmol/L EDTA with protease inhibitors (1 mmol/L sodium vanadate, 0.01 mg/mL leupeptin, 0.04 mg/mL aprotinin, 20 mg/mL sodium fluoride, 0.01 mg/mL phenylmethylsulfonyl fluoride). Frozen tissue was ground in liquid nitrogen and homogenized in ice with lysis buffer with the use of 20 μL of lysis buffer per 1 mg of tissue. After 30 minutes on ice, the samples were centrifuged at 14 000g for 10 minutes at 4°C . Supernatant was transferred to new tubes, and total protein content was determined by a detergent-compatible assay (DC Protein Assay, Bio-Rad). Equal amounts of total protein (60 μg) were loaded for each lane on 10% SDS-PAGE. Commercially available antibodies (Chemicon International, Temecula, Calif) used were mouse anti-human integrin $\beta 1\text{D}$ monoclonal antibody (specific for the cytoplasmic domain of integrin $\beta 1\text{D}$ subunit), mouse anti-human integrin $\beta 3$ monoclonal antibody, rabbit anti-human

ADAM10 polyclonal antibody, anti-ADAM12 polyclonal antibody, rabbit anti-ADAM15 polyclonal antibody, and anti-tumor necrosis factor- α (TNF- α) converting enzyme (TACE)/ADAM17 polyclonal antibody. In addition, we used TIMP-3 monoclonal antibody (OncoGene Research Products). Membranes were developed with ECL reagent (Amersham Pharmacia) and quantified by image analysis. The results were presented as percent change compared with NF controls, and their means were arbitrarily set as 100%.

Integrin Cleavage Products

With the use of the immunoblots for integrin $\beta 1\text{D}$ as described above, the integrin $\beta 1\text{D}$ cleavage ratio was calculated by measuring the relative expression of the intact mature protein (140-kDa band) to its primary cleavage product in the same lane (40-kDa band). The cleavage ratio was calculated for each sample with the 40-kDa "cleavage" band used as the numerator and the 140-kDa "intact" band used as the denominator.

Confocal Microscopy of ADAM and Integrin Expression

To localize and quantify regional protein expression, double immunofluorescent stainings were done on 15- to 20- μm -thick frozen sections from LV segments. Frozen slides were allowed to equilibrate to room temperature before they were fixed in ice-cold acetone for 2 minutes. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide followed by blocking of nonspecific binding sites with 10% normal goat serum. Slides were then treated with various combinations of primary antibody (as performed for immunoblotting) cocktails that are raised in different species. Fluorochrome-conjugated secondaries (Alexa-Fluors, Molecular Probes) were used to enhance the signal. Nuclear counterparts were stained with Topro-3 (Molecular Probes). Sections were imaged with the use of a laser scanning confocal system (model MRC 1024, Bio-Rad). Fields were randomly selected with the exclusion of cutting or compression artifact. Four random fields were obtained from each of 2 transverse LV segments from each of 5 patient samples per group. The stained LV sections were then digitized at a final magnification of $\times 1200$ and analyzed with an image analysis system (Scion Image, NIH Software). Image analysis was performed to quantify these parameters in blinded fashion.

Statistical Analysis

Results are presented as mean \pm SD unless otherwise specified. Comparison between groups was performed by 1-way ANOVA. When significant, pairwise tests of individual group means were compared with the Student-Newman-Keuls test. Paired samples obtained at LVAD insertion/transplantation were analyzed by paired t test. All statistical procedures were performed with the SAS software system (SAS Institute). Differences were considered significant at $P < 0.05$.

Results

Patient Profiles

Baseline patient characteristics are summarized in Table 1. Our study group was composed of patients with DCM ($n=20$), patients with HOCM ($n=5$), and patients with structurally normal and nonfailing hearts collected at the time of organ procurement for transplantation (NF group) ($n=7$). Patients with DCM had significantly enlarged LVs (mean LV end-diastolic diameter [LVEDD], 7.2 ± 0.9 cm; $P < 0.001$) and reduced ventricular function (mean ejection fraction as percentage [%EF], 13 ± 4 ; $P < 0.001$) compared with the patients with HOCM (mean LVEDD, 4.5 ± 0.3 cm; mean %EF, 65 ± 4) or NF hearts (mean LVEDD, 4.7 ± 0.8 cm; mean %EF, 56 ± 4). DCM patient samples were obtained at the time of cardiac transplantation. Serial LV myocardial samples were obtained in 10 patients with DCM at the time of LVAD insertion and repeated at the time of transplantation during LVAD removal. Mean duration of LVAD

TABLE 1. Patient Characteristics and Echocardiographic Data

ID	Age, y	Sex	Race	Diagnosis	Rhythm	VAD Duration, Days of Support	Drugs	LVEDD, cm	LVEDS, cm	IVS, cm	PW, cm	%EF
F1	59	M	W	DCM	AF		Amiodarone, captopril, digoxin, MT	5.2	4.4	1.0	1.1	25
F2	51	F	W	DCM	AF		Amiodarone, captopril, digoxin, dobutamine	6.6	6.2	0.8	1.3	10
F3	18	M	W	DCM	SR		Digoxin, dobutamine, enalapril, milrinone, carvedilol					
F4	52	M	W	DCM	SR		Amiodarone, captopril, milrinone	6.1	5.7	0.8	1.0	20
F5	65	M	W	DCM	SR		Amiodarone, dobutamine	7.5	6.7	1.0	1.0	10
F6	45	M	W	DCM	SR		Amiodarone, captopril, milrinone	8.1	7.1	0.8	0.9	10
F7	48	F	W	DCM	AF		Amiodarone, captopril, digoxin, MT	7.1	6.2	0.9	1.0	10
F8	34	F	W	DCM	SR		Amiodarone, captopril, digoxin, dobutamine	6.2	5.8	0.8	1.0	20
F9	41	M	W	DCM	SR		Amiodarone, captopril, milrinone	8.0	7.0	0.9	0.9	10
F10	39	M	W	DCM	SR		Amiodarone, captopril, dobutamine	7.6	5.8	1.2	1.2	15
H1	69	M		HOCM	SR			4.1	2.9	2.2	1.3	60
H2	20	M	W	HOCM	SR			4.5	2.2	2.9	1.4	65
H3	35	M	W	HOCM	SR			4.9	2.2	2.4	1.5	65
H4	64	F	W	HOCM	SR							70
H5	64	F	W	HOCM	SR			4.6	2.1	1.8	1.5	60
NF1	61	F	W	CVA	SR		Dopamine, norepinephrine bitartrate, pitressin	3.6	2.8	0.7	0.7	50
NF2	32	F	W	GSW	SR		Norepinephrine, CP, PT					60
NF3	48	F	W	CVA	SR		Dopamine	5.5	3.5	1.0	0.9	55
NF4	42	M	W	MVA	SR		Dopamine, phenylephrine					
NF5	53	M	W	CVA	SR		Dopamine, norepinephrine, thyroxine					55
NF6	45	M	W	MVA	SR		Dopamine	4.7	2.0	1.1	1.0	55
NF7	33	M	W	MVA	SR		Dopamine	4.8	2.1	1.0	0.9	60
A	62	M	W	DCM	SR	27	Dobutamine (milrinone)	7.0				15
B	40	M	W	DCM	SR	340	Digoxin, dobutamine, milrinone (amiodarone)	6.0	4.9	1.1	1.1	15
C	56	F	W	DCM	SR	130	Amiodarone, digoxin, milrinone (amiodarone, losartan)	7.6	6.9			10
D	43	M	B	DCM	SR	19	Dobutamine, dopamine (amiodarone, dobutamine, enalapril, milrinone)	7.6	5.8	1.2	1.2	15
E	62	M	W	DCM	SR	33	Amiodarone, dobutamine, milrinone, norepinephrine (amiodarone)	8.7	7.2	0.9	0.9	10
F	52	M	B	DCM	SR	96	Amiodarone, digoxin, dobutamine, milrinone (amiodarone)	6.6	6.4	1.1	1.1	10
G	60	M	W	DCM	SR	26	Amiodarone, dobutamine, milrinone (amiodarone, milrinone)	8.3	8.1	1.0	1.1	15
H	53	M	W	DCM	SR	27	Captopril, enalapril (amiodarone, milrinone)	7.5	7.0	0.8	0.9	15
I	21	M		DCM	SR	45	Dobutamine, dopamine, norepinephrine bitartrate, amiodarone, captopril	7.8	6.7	0.9	0.9	10
J	30	M	W	DCM	SR	28	Amiodarone, captopril, dobutamine (amiodarone)	6.5	5.9	1.0	0.9	10

Echo data are taken from the last echo before surgery, all within 30 days. LVEDD indicates LV end-systolic diameter; PW, posterior wall thickness; W, white; B, black; MT, metoprolol tartrate; CVA, cerebrovascular accident; GSW, gunshot wound; MVA, motor vehicle accident; AF, atrial fibrillation; and SR, sinus rhythm. The drugs in parentheses are additional drugs that were added after LVAD insertion.

support for these patients was 77 ± 99 days (range, 19 to 340 days). HOCM tissue samples were obtained during surgical resection of the obstructed LV outflow tract (peak gradient >75 mm Hg in all cases), and, accordingly, interventricular septal wall thickness (IVS) was significantly greater in HOCM patients (mean IVS, 2.3 ± 0.5 cm; $P < 0.05$) than in DCM (mean IVS, 1.0 ± 0.1 cm) and NF patients (mean IVS, 1.0 ± 0.2 cm).

Differential ADAM Expression

The differential profile of ADAM protein expression (ADAM10, ADAM12, ADAM15, ADAM17) was assessed in DCM, HOCM, and NF myocardium by means of immunoblotting with commercially available antibodies and quantification by computerized image analysis. Baseline expression of each

ADAM studied was consistently observed in NF myocardium, suggesting constitutive expression. Notably, there was a unique pattern of ADAM expression that followed the underlying pattern of cardiac remodeling. In the dilated and dysfunctional hearts (DCM), ADAM10 and ADAM15 were uniquely increased (Figure 1). In contrast, ADAM12 was markedly increased in HOCM patients (Figure 2). ADAM17 expression was similarly increased in all remodeled hearts (DCM and HOCM) compared with NF hearts (Figure 2). In addition, we assessed ADAM expression in the serial samples obtained before and after LVAD support for DCM patients. These results are summarized in Table 2. Notably, expression of ADAM10 and ADAM12 was significantly reduced after hemodynamic unloading by LVAD support.

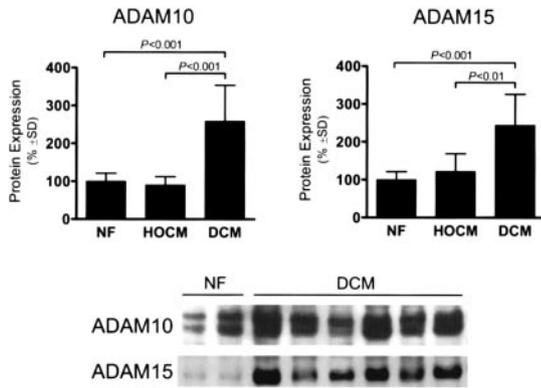


Figure 1. ADAM10 and ADAM15 protein expression. A unique pattern of ADAM protein expression was observed in human myocardium that reflects the underlying state of structural cardiac remodeling. ADAM10 and ADAM15 were uniquely increased in DCM patients compared with NF hearts. The 62-kDa bands for mature ADAM10 and ADAM 15 are shown above and were used for calculation of protein expression.

Expression of the Endogenous ADAM Inhibitor TIMP-3

TIMP-3 is expressed in human myocardium and serves as the primary endogenous inhibitor of ADAM10, ADAM12, and ADAM17. Accordingly, we assessed TIMP-3 expression in our patient samples by immunoblotting and quantitative computerized image analysis. These results are summarized in Figure 3. TIMP-3 expression was reduced in dilated failing hearts (DCM) compared with nondilated hearts with preserved systolic function (HOCM and NF). By confocal microscopy, TIMP-3 expression was localized to cardiomyocytes. Confirming the immunoblot data, TIMP-3 expression by confocal microscopy was also observed to be uniquely reduced in the DCM samples (Figure 3, bottom panel).

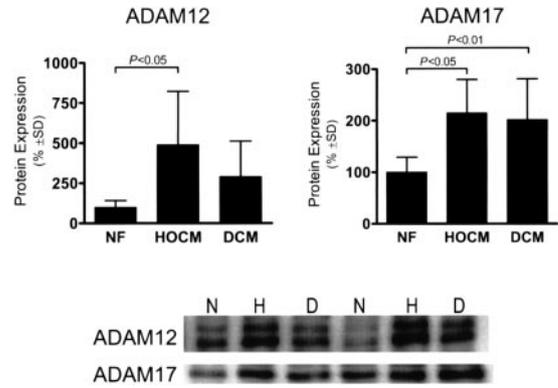


Figure 2. ADAM12 and ADAM17 protein expression. ADAM12 was markedly increased in HOCM patients. ADAM17 expression was similarly increased in all remodeled hearts (DCM and HOCM) compared with NF hearts. The 80-kDa band for ADAM12 and 85-kDa band for ADAM17 were assessed and are shown in a representative immunoblot (bottom). NF or N indicates nonfailing; HOCM or H, hypertrophic obstructive cardiomyopathy; and DCM or D, dilated cardiomyopathy.

Integrin Receptor Expression and Turnover

We assessed the expression and turnover of 2 key integrin receptors, integrin β 1D and integrin β 3. These results are summarized in Figure 4. Notably, intact integrin β 1D protein expression was increased in HOCM hearts but not in dilated failing hearts (DCM). However, integrin cleavage products were increased in DCM hearts, suggesting accelerated breakdown. ADAM10 ($r=0.58, P=0.02$) and ADAM15 expressions ($r=0.64, P=0.01$) were found to be positively correlated with the presence of integrin β 1D cleavage products. By confocal microscopy, ADAM10 expression appeared to colocalize with integrin β 1D at the cardiomyocyte cell surface, where shedding may occur (Figure 5).

In patients with DCM, hemodynamic unloading of the heart by LVAD support resulted in a recovery of integrin β 1D

TABLE 2. Effects of LVAD Support in End-Stage DCM

Patient	ADAM10	ADAM12	ADAM15	ADAM17	TIMP-3	β 1D	β 3
A	68	64	100	63	75	130	110
B	82	75	85	81	100	168	92
C	63	63	93	59	71	156	98
D	88	53	50	77	124	194	123
E	100	58	61	100	90	130	89
F	107	71	76	35	96	180	98
G	91	60	175	153	105	97	91
H	93	67	50	183	105	163	97
I	100	90	67	223	148	442	103
J	70	48	75	89	113	119	105
Mean	86.2	64.9	83.2	106.3	102.7	177.9	100.6
SD	15.0	11.9	36.3	60.3	22.6	97.4	10.2
P	0.02	<0.001	0.18	0.75	0.71	0.03	0.86

Ten paired myocardial samples taken at the time of LVAD insertion for end-stage DCM were assessed for ADAM, integrin, and TIMP-3 protein expression and compared after LVAD removal at the time of heart transplantation. Data are expressed as percent change compared with patient's own expression level at the time of LVAD insertion. ADAM10 and ADAM12 expression were significantly reduced after hemodynamic unloading by LVAD support with a corresponding recovery of integrin β 1D expression. ADAM15 expression was similarly reduced, although the difference was not statistically significant. P value was obtained from paired t test.

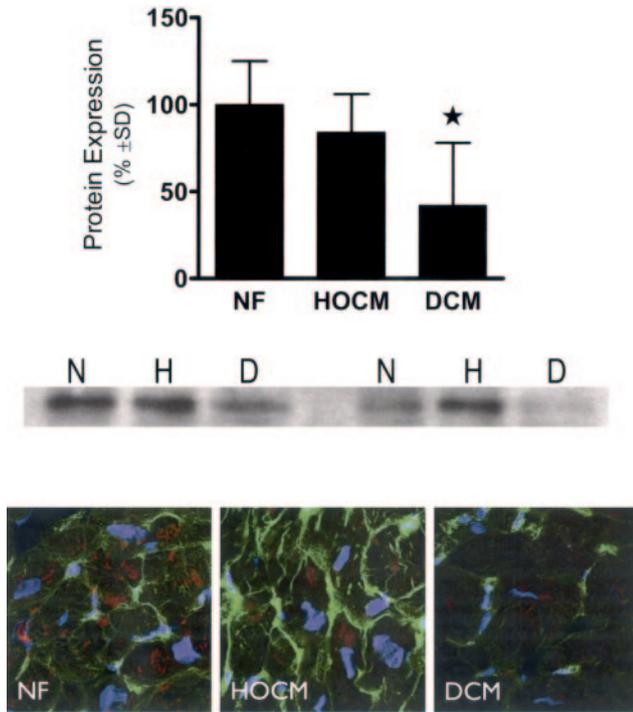


Figure 3. Expression of the endogenous disintegrin metalloproteinase inhibitor TIMP-3. TIMP-3 protein expression as assessed by immunoblotting (top and middle) and confocal microscopy (bottom) is shown. TIMP-3 expression was reduced in dilated failing hearts (DCM) compared with nondilated hearts with preserved systolic function (HO and NF). The 30-kDa glycosylated band for TIMP-3 was assessed and is shown in a representative immunoblot (middle). By confocal microscopy, TIMP-3 expression was also reduced in DCM and localized to cardiomyocytes (bottom). Abbreviations are as defined in Figure 2 legend. * $P < 0.05$.

expression, whereas integrin $\beta 3$ expression was unchanged (Table 2). Increased integrin $\beta 1D$ expression was observed with a concomitant decrease in expression of ADAM10 and ADAM12. ADAM15 expression was similarly reduced, although the difference was not statistically significant.

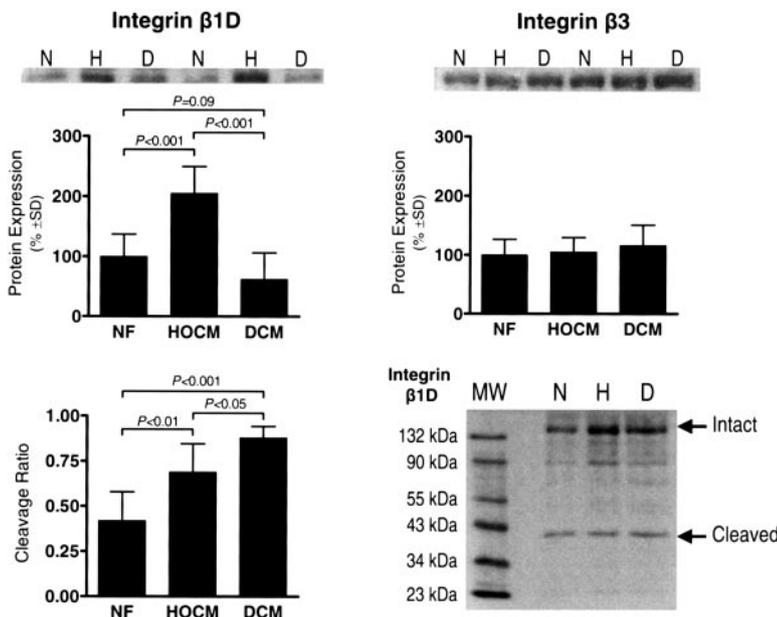


Figure 4. Integrin receptor expression and cleavage pattern. Integrin $\beta 1D$ receptor expression and turnover were altered with structural cardiac remodeling, whereas integrin $\beta 3$ was unchanged. Integrin $\beta 1D$ was assessed by a 140-kDa band on immunoblotting (top left), and integrin $\beta 3$ (top right) was assessed by a 104-kDa band as shown in representative immunoblots. The cleavage ratio was calculated for integrin $\beta 1D$ and compared between groups (bottom right). The cleavage pattern for integrin $\beta 1D$ is also shown on a representative immunoblot (bottom right). The cleavage ratio was calculated as the ratio of “cleaved” protein at 40 kDa relative to its “intact” protein at 140 kDa in the same lane. MW indicates molecular weight. Other abbreviations are as defined in Figure 2 legend.

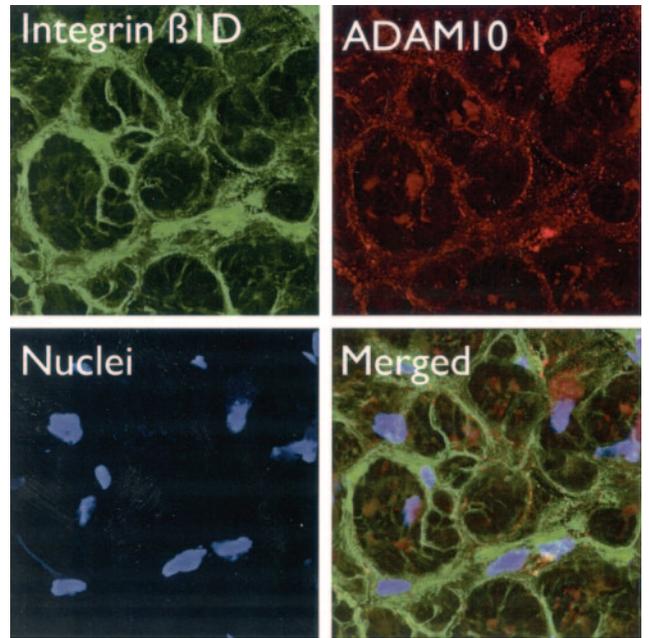


Figure 5. ADAM10 colocalizes to integrin $\beta 1D$ in cardiomyocytes. With the use of confocal microscopy, ADAM10 (red) colocalized to cell surface integrin $\beta 1D$ receptors (green) in human myocardium. Integrin $\beta 1D$ served as a myocyte-specific marker because it is only expressed by cardiomyocytes in heart tissue.

Confocal Microscopy

Confocal microscopy was performed for ADAM10, ADAM12, ADAM17, and integrin $\beta 1D$. In all images, 3 channels were used to simultaneously highlight the cell nucleus, ADAM of interest, and coexpression of integrin $\beta 1D$. In heart tissue, integrin $\beta 1D$ is only expressed by cardiomyocytes, and, accordingly, its expression also served as a cardiomyocyte-specific marker. Computerized image analysis of multiple images for each sample with the use of a single channel allowed a quantitative analysis of individual protein expression. Representative confocal images are displayed in Figure 6. Following the

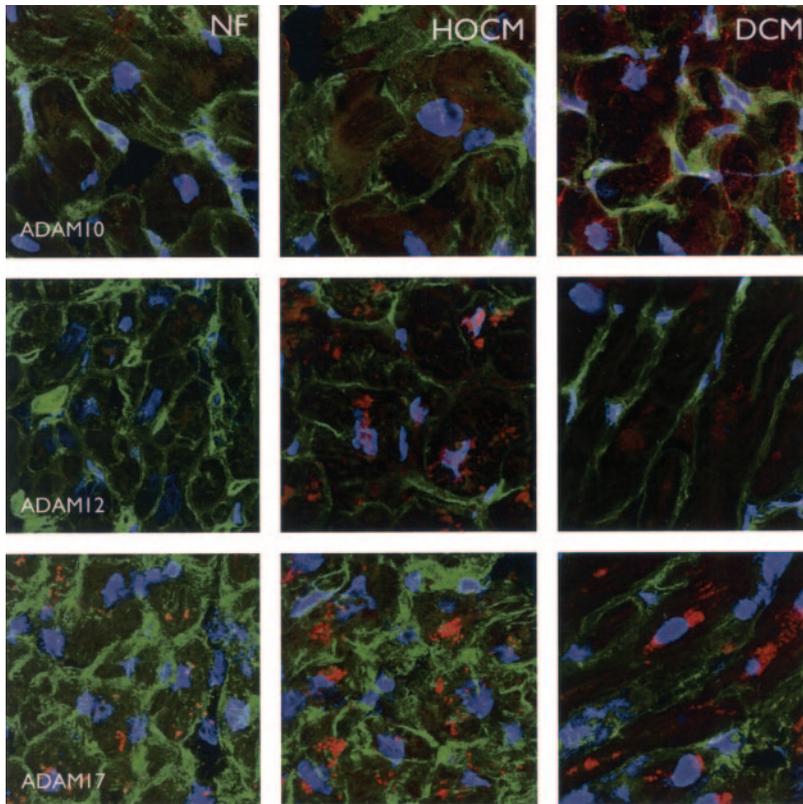


Figure 6. Confocal microscopy of ADAM and integrin expression in human myocardium. Confocal microscopy was performed for ADAM10, ADAM12, ADAM17, and integrin β 1D. In all images, 3 channels were used to simultaneously highlight the cell nucleus (blue), ADAM of interest (red), and coexpression of integrin β 1D (green). Computerized image analysis of multiple images for each sample with the use of a single channel allowed a quantitative analysis of individual protein expression. Notably, the expression of ADAM proteins and integrin β 1D followed the pattern of expression observed with immunoblotting.

pattern of expression with immunoblotting, ADAM10 expression as assessed by confocal microscopy was significantly increased compared with NF controls (19.2 ± 15 versus 6.1 ± 3 arbitrary units; $P=0.002$). In addition, ADAM10 expression was positively correlated with LVEDD ($r=0.52$, $P=0.03$) and LVESD ($r=0.63$, $P=0.007$) and negatively correlated with %EF ($r=-0.67$, $P=0.002$).

Discussion

Structural cardiac remodeling is the reorganization of heart size and shape that underlies heart failure progression. Ventricular dilatation is a major determinant of the clinical course of congestive heart failure irrespective of its underlying cause. To define new therapeutic targets that control the progression of heart failure, novel biomolecules and mechanisms that regulate structural cardiac remodeling must be discovered. To this end, dysregulation of the interstitial myocardial matrix has emerged as a key determinant of ventricular dilatation and dysfunction. An imbalance of MMPs and their endogenous inhibitors (TIMPs) has been identified in failing hearts and is believed to promote ventricular dilatation by degrading the matrix scaffold that supports functional tissue architecture.² Disintegrin metalloproteinases (ADAMs) are a unique class of MMP proteases expressed in human myocardium. ADAM enzymes may be involved in structural remodeling given their documented ability to cleave and activate growth factors and cytokines. In addition, ADAMs may regulate cell-cell or cell-matrix adhesions via proteolytic activity or by interactions between their disintegrin domains and integrin proteins resulting in structural tissue remodeling. However, ADAM enzymes have not

been adequately studied in the failing human heart, and their role in the myocardium is largely unclear.

We previously demonstrated that the endogenous metalloproteinase inhibitor TIMP-3 is deficient in failing myocardium and its deficiency parallels the progression of matrix and structural cardiac remodeling.³ We extended these observations in mutant mice lacking the ability to express TIMP-3 by demonstrating that TIMP-3 deficiency directly results in progressive DCM.⁴ At the molecular level, TIMP-3 deficiency resulted in MMP-mediated matrix degradation and ADAM-mediated inflammatory cytokine activation. Notably, TIMP-3 is a potent endogenous inhibitor of disintegrin metalloproteinases, particularly ADAM10, ADAM12, and ADAM17.⁵⁻⁸ In light of the emerging relationship between loss of myocardial TIMP-3 and progressive structural remodeling, we considered these additional targets of TIMP-3 as contributors to the remodeling process. Accordingly, we surmised that a loss of TIMP-3 in human heart failure may release ADAM activity, contributing to structural remodeling. However, the profile of expressed ADAMs in human heart tissue and the relationship of these enzymes to the underlying state of cardiac remodeling had not been outlined before this study.

Unique Pattern of ADAM Expression in DCM

The present study indicates that ADAM enzymes are differentially expressed in human myocardium, and the pattern of expression follows the underlying pattern of structural remodeling. Although the specific role of each ADAM in cardiac remodeling remains undefined, there appears to be a characteristic expression of ADAM proteins in human DCM. ADAM10 and ADAM15 were exclusively elevated in human DCM. In support of a role in structural remodeling, ADAM10 expression

was highly correlated with LV dilatation and indices of systolic contractile dysfunction. Interestingly, these specific disintegrin metalloproteinases have been similarly implicated in human atrial dilatation.⁹ Increased expression of ADAM10 and ADAM15 was associated with an abundance of integrin β 1D cleavage products, suggesting a role for these enzymes in integrin β 1D receptor shedding and turnover. Further supporting this mechanism, ADAM10 was colocalized to integrin β 1D expression by confocal microscopy. Other studies have demonstrated a colocalization of ADAM15 with integrin β 1D expression in cardiac cells.⁹ Transgenic mice engineered to lack cardiac expression of integrin β 1D develop a spontaneous DCM, supporting a mechanistic role for integrin β 1D loss and cardiac dilatation.¹⁰ In summary, the data from our study suggest that increased expression of ADAM10 and ADAM15 may play a role in human cardiac dilatation and failure by reducing cell-matrix interactions via integrin β 1D shedding. Further studies will be required to confirm the validity of this proposed mechanism.

Hypertrophic Hearts and ADAM12 Expression

ADAM12 may influence muscle growth by stimulating key growth factors. In transgenic mouse models, ADAM12 activity directly triggered cardiomyocyte hypertrophy by activating heparin-binding epidermal growth factor.⁸ In addition, ADAM12 can stimulate the insulin-like growth factor-1 pathway,⁵ a growth factor strongly implicated in human hypertrophic cardiomyopathy.¹¹ In support of this mechanism, ADAM12 expression was profoundly increased in patients with HOCM. In the dilated but not thickened ventricles of patients with DCM, ADAM12 was not similarly elevated. Interestingly, HOCM patients had an increased expression of integrin β 1D with a concomitant increase in the cleavage ratio. These data suggest increased integrin expression with simultaneous increased degradation. This finding is in contrast to those in DCM patients who were unable to maintain adequate levels of this key cell-matrix receptor. In noncardiac cells, ADAM12 has been observed to alter cell shape by regulating integrin β 1D function.¹² Specifically, ADAM12 binds and cleaves integrin β 1D, resulting in altered cell morphology, differentiation, and survival. These data may explain the increased integrin cleavage observed in HOCM patients without concomitant increases in ADAM10 and ADAM15. Cell-matrix interactions may be important to maintain cell and chamber hypertrophy, and a dynamic turnover may be required to support an ongoing remodeling process in hypertrophic tissue.

ADAM17, TNF- α , and Inflammatory Pathways

Congestive heart failure is increasingly attributed to inflammatory processes. ADAMs shed and activate inflammatory cytokines and growth factors.^{13,14} Activation of TNF- α is mediated by ADAM17, also known as TACE.¹⁵ Structural cardiac remodeling (both cellular hypertrophy and chamber dilatation) is associated with elevated TNF- α activity. For example, overexpression of TNF- α in an animal model is associated with matrix proteolysis, cell death, and dilated cardiac failure.¹⁶ In addition, ADAM17 activity is strongly associated with ventricular dilatation and cardiac dysfunction

in human heart failure.¹⁷ It is not surprising that ADAM17 was elevated in both DCM and HOCM hearts given the ubiquitous nature of proinflammatory cytokines and tissue remodeling. Similarly, given the inflammatory pathways activated by artificial mechanical hearts, LVAD support did not reduce ADAM17 expression.

Reverse Remodeling and Mechanical Unloading of the Failing Heart

Mechanical unloading of the dilated failing heart by LVAD support can promote reverse remodeling, an intriguing phenomenon that may provide novel insights toward mechanisms of myocardial recovery. We observed a recovery of ADAM expression after mechanical unloading in patients with DCM (Table 2). ADAM10 was significantly reduced after mechanical unloading, and ADAM15 was similarly reduced, although the difference was not statistically significant. Integrin β 1D expression was profoundly increased with mechanical support, perhaps a result of lessened degradation from attenuated ADAM activity. Given that chamber size is sometimes reduced after mechanical unloading of the dilated failing heart, one may speculate that this is a consequence of normalized ADAM activities and preserved cell-matrix contacts. Interestingly, mechanical unloading profoundly reduced the expression of ADAM12 in DCM, suggesting that ADAM12 expression may be linked to wall stress and mechanical stretch. Given that ADAM12 triggers myocardial hypertrophy, this observation may provide some mechanistic insight into the upstream signals regulating this novel muscle growth pathway.

Deficient Endogenous ADAM Inhibitor TIMP-3 as a Therapeutic Target

In support of previous studies, TIMP-3 was reduced in DCM. Given its role in regulating the activity of the specific ADAMs studied, we speculate that the loss of this critical regulator combined with the upregulation of ADAM10 and ADAM15 resulted in excessive and maladaptive ADAM activity in the DCM heart. Notably, we did not observe a recovery of TIMP-3 expression after mechanical unloading of the dilated heart, despite the results of prior studies.¹⁸ Given the observations of this study, TIMP-3 may reflect an important therapeutic target in the quest to halt the progression of structural cardiac remodeling. Targeting disintegrin metalloproteinases, perhaps by restoring deficient TIMP-3 levels with gene or cell-based therapies, may prevent progressive chamber dilatation in human DCM.

Limitations

First, human tissue samples were harvested from the LV but, in some cases, from different regions within the LV (ie, HOCM tissue from the outflow tract versus DCM tissue from the anterior free wall). Regional differences in expression within the LV could result from regional differences in wall stress. Further studies will be required to determine the regional and load-dependent expression of disintegrin metalloproteinases in the human ventricle. Second, the specific β 1D cleavage products that may result from ADAM interactions remain undefined. Further characterization of these cleavage products will be required to demonstrate the potential role of ADAM enzymes in the process of integrin shedding and cardiac structural remodeling.

Conclusion

This study documents the novel observation that disintegrin metalloproteinases are differentially expressed in human myocardium in a pattern that reflects the underlying structural remodeling. ADAM10 and ADAM15 may contribute to progressive cardiac dilatation by reducing cell-matrix interactions via integrin shedding. ADAM12 may play an important role in myocardial hypertrophy. ADAM17 may regulate inflammation in the process of cardiac remodeling. In conclusion, disintegrin metalloproteinases may reflect an important new therapeutic target for the prevention of congestive heart failure.

Acknowledgments

This study was supported by the Heart and Stroke Foundation of Ontario (NA 5299 and T5206) and the Canadian Institutes for Health Research (MOP 62698 and MOP 14795). Dr Li is a Career Investigator of the Heart and Stroke Foundation of Canada.

Disclosures

None.

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CLINICAL PERSPECTIVE

Structural cardiac remodeling, particularly ventricular dilatation, is the hallmark of congestive heart failure. Hemodynamic unloading by mechanical assistance (left ventricular assist device) results in a reversal of structural remodeling in some patients. Defining the molecular mechanisms underlying structural cardiac remodeling and its reversal may result in novel therapeutic targets for the growing population of patients with heart failure. Altered interactions between cells and their interstitial (extracellular) matrix may play a key role in structural cardiac remodeling. Disintegrin metalloproteinases are a unique group of endogenous proteases capable of altering cell-surface matrix receptors (integrins). We examined human heart specimens with characteristic patterns of structural cardiac remodeling (dilated cardiomyopathy, hypertrophic cardiomyopathy, and nonfailing controls) and profiled the expression of disintegrin metalloproteinases, their endogenous inhibitor tissue inhibitor of metalloproteinases-3, and key integrin receptors. We obtained serial heart specimens from patients with dilated heart failure before and after left ventricular assist device support to explore “reverse remodeling.” We determined that disintegrin metalloproteinases are differentially expressed in human myocardium in a profile that reflects the underlying pattern of structural remodeling. Our data suggest that specific disintegrin metalloproteinases disrupt cell-matrix interactions and contribute to ventricular dilatation. Targeting disintegrin metalloproteinases, perhaps by inhibiting their activity with gene or cell-based therapies, may prevent progressive chamber dilatation and failure in human dilated cardiomyopathy.