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Loss of a gp130 Cardiac Muscle Cell Survival Pathway Is a Critical Event in the Onset of Heart Failure during Biomechanical Stress

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Summary

Biomechanical stress is a major stimulus for cardiac hypertrophy and the transition to heart failure. By generating mice that harbor a ventricular restricted knockout of the gp130 cytokine receptor via Cre-loxP-mediated recombination, we demonstrate a critical role for a gp130-dependent myocyte survival pathway in the transition to heart failure. Such conditional mutant mice have normal cardiac structure and function, but during aortic pressure overload, these mice display rapid onset of dilated cardiomyopathy and massive induction of myocyte apoptosis versus the control mice that exhibit compensatory hypertrophy. Thus, cardiac myocyte apoptosis is a critical point in the transition between compensatory cardiac hypertrophy and heart failure. gp130-dependent cytokines may represent a novel therapeutic strategy for preventing in vivo heart failure.

Introduction

Cytokines play a critical role in the control and maintenance of signaling pathways that regulate mammalian physiology in multiple organ systems. Their widespread importance is reflected in the extensive tissue distribution of the cytokine network, their pleiotropic effects in the in vivo setting, the existence of conserved pathways of signal transduction, and a large degree of functional redundancy in a variety of organ systems (reviewed in Kishimoto et al., 1994; Taga and Kishimoto, 1997). For example, mice that harbor a complete deficiency in individual members of the interleukin 6 (IL-6) family of cytokines (IL-6, leukemia inhibitory factor [LIF], ciliary neurotrophic factor [CNTF], or IL-11 receptor α) or their downstream gp130-dependent signaling components can display multiple organ defects, including disorders of the immune system, hepatic function, bone metabolism, neurological function, and hematopoiesis (Stewart et al., 1992; Bernad et al., 1994; Kopf et al., 1994; Poli et al., 1994; Ramsay et al., 1994; Cressman et al., 1996; Romani et al., 1996; Klein et al., 1997; Nandurkar et al., 1997; Romano et al., 1997). Embryos that harbor a complete deficiency in the common cytokine signal transducer gp130 display embryonic lethality and defects in diverse embryonic compartments (Yoshida et al., 1996). Similarly, mice that lack the LIF receptor (LIFR), the shared component of the receptor complexes for LIF, cardiotrophin-1 (CT-1), and CNTF, die in the perinatal window of development and exhibit placental, skeletal, neural, and metabolic defects (Li et al., 1995; Ware et al., 1995). Unfortunately, given the pleiotropic effects of the IL-6 family on multiple tissues, and the genetic ablation of these widely expressed components of the cytokine signaling pathway in virtually every cell type, it is often difficult to conclude from these studies whether these defects arise as a result of a primary or secondary requirement for a specific cytokine signaling pathway.

Recently, this point has become of particular importance in elucidating the specific role of the IL-6-related cytokines in the pathogenesis of cardiac failure, the leading cause of combined morbidity and mortality in the U.S. and other developed nations. In response to chronic increases in hemodynamic pressure (e.g., long-standing hypertension) and volume overload (e.g., postmyocardial injury), the heart initiates an adaptive response of compensatory hypertrophy that leads to cardiac enlargement and a maintenance of normal cardiac function. However, there can be a temporal transition under conditions of chronic stimulation where these biomechanical stimuli subsequently activate pathways that lead to cardiac muscle cell dysfunction, myocyte loss, replacement fibrosis, and overt congestive heart failure (for a review, see Chien et al., 1998). A growing body of evidence suggests the possibility that cytokines which operate via gp130 pathways might play a critical role in the onset of cardiac failure (for a review, see Wollert and Chien, 1997). Previous studies coupling an in vitro embryonic stem cell model of cardiogenesis (Robbins et al., 1990; Miller-Hance et al., 1993) and expression cloning with a high-throughput cardiac myocyte hypertrophy assay system (Shubeita et al., 1990; Pennica et al., 1995) resulted in the isolation of a new member of the IL-6 cytokine family, CT-1 (Pennica et al., 1995; Sheng et al., 1996; Wollert et al., 1996). CT-1 can activate several features of myocyte hypertrophy in vitro, including cell enlargement without proliferation, sarcomeric organization, and embryonic gene expression. In addition, CT-1 is a potent myocyte survival factor for differentiated cardiac muscle cells (Sheng et al., 1997) and is capable of blocking the onset of cardiomyocyte apoptosis following serum deprivation (Sheng et al., 1997). Recent findings indicate that the widespread, constitutive expression of gp130 in transgenic mice can lead to cardiac hypertrophy (Hirota et al., 1995). In addition, embryonic cardiac defects are seen in completely deficient gp130

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mutant embryos that likely lead to early lethality (Yoshida et al., 1996). However, these studies do not necessarily reflect the normal, primary effect of gp130 on in vivo cardiac function, given that the nature of the genetic manipulation was not restricted to the cardiac compartment, and other noncardiac defects were present in each case.

Using Cre-lox technology to achieve a ventricular chamber–restricted knockout of the gp130 cytokine receptor, we now provide unequivocal evidence for a critical role of a gp130-dependent myocyte survival pathway. These studies identify cardiac myocyte apoptosis as a critical point in the transition between compensatory cardiac hypertrophy and heart failure and suggest that ligands operating via gp130 may represent a novel therapeutic strategy for preventing heart failure in the in vivo context. This study represents clear evidence that promoting cardiac myocyte survival is a critical event in the transition between compensatory hypertrophy and the onset of heart failure in the in vivo context.

Results

Generation of Ventricular Muscle–Specific gp130 Knockout Mice

To generate mice that harbor a ventricle chamber restricted knockout of the *gp130* gene, we utilized Cre-lox strategies, employing *gp130* floxed allele mice (Betz et al., 1998). This floxed *gp130* allele contains two loxP sites that have been introduced into exon 16, which encodes the transmembrane domain of gp130 (designated "*gp130flox*") (Betz et al., 1998) (Figure 1A). Following the delivery of Cre, this critical exon is excised, leading to the loss of gp130 from the plasma membrane, resulting in a complete lack of responsiveness to gp130-related cytokines. To mutate this allele exclusively in ventricular muscle cell lineages, we have employed a knock-in (KI) strategy and homologous recombination to insert Cre coding sequences into the genomic locus of the myosin light chain 2v (*MLC2v*) gene (designated "*MLC2vCreKI*") (Chen et al., 1998a, 1998b), which is the earliest ventricular-restricted marker during mammalian cardiogenesis (O'Brien et al., 1993; Ross et al., 1996). We have previously reported that matings between these *MLC2v* Cre knock-in mice and an *RXR*α floxed allele can generate a high-efficiency cardiac ventricular muscle knockout at E8.75 with no detectable effect on atrial muscle. The generation and characteristics of the *MLC2v* knock-in mice have been previously described in detail elsewhere (Chen et al., 1998a, 1998b).

Accordingly, we bred *gp130flox/flox:MLC2v+/+* and *gp130flox/+:MLC2vCreKI/+* mice, resulting in *gp130flox/flox:MLC2vKI/+* mice, which harbor a ventricle-specific gp130 knockout (GP CKO). Adult ventricular tissue and tails from GP CKO mice were extracted and genomic DNA digested and probed to visualize the nonrecombined floxed allele and the recombined floxed-out allele. To assess the efficiency of Cre recombination in ventricular muscle, we purified ventricular myocytes from GP CKO mice. As shown in Figure 1B, 70% of the alleles in the heart from GP CKO mice have undergone recombination, while no evidence of the floxed out allele is found in tail DNA, demonstrating tissue specificity of the floxed out allele. To demonstrate that a deficiency in gp130 had been established, we directly examined the level of gp130 by quantitative Western blotting; the immunoblot (shown in Figure 1C) was probed with an antibody to gp130, thereby documenting the reduction of the gp130 protein (60%) exclusively in the heart of GP CKO mice, with no detectable decrease in the liver. In addition, there was almost no difference in cardiac levels of gp130 in studies of other genotypes, using *gp130flox/+*, *MLC2vCreKI/+* (CNT) as a control. When cardiomyocytes were isolated as a partially purified single cell preparation, the ratio of the gp130 protein level in cardiomyocytes from GP CKO versus CNT mice was approximately 20% (Figure 1D), indicating that there was at least an 80% decrease in gp130 protein levels in ventricular muscle cells. In Figure 1C and 1D, the expression of STAT3 was comparable between these groups, thereby demonstrating specificity of the gp130 decrease.

The Requirement for gp130 during Cardiac Development Is Non–Cardiac Muscle Autonomous

Surprisingly, GP CKO mice have normal embryonic viability without evidence of any of the cardiac morphogenic defects observed in the complete gp130-deficient embryos (Yoshida et al., 1996) and the mice with postnatally inactivated gp130 (Betz et al., 1998). Pups of GP CKO mice were born normally and externally indistinguishable from littermates of other genotypes. The GP CKO mice were recovered at Mendelian frequency and grew to adulthood. Histological examination of GP CKO hearts at 8 weeks revealed no evidence of necrosis, ventricular fibrosis, or myofibrillar disarray (data not shown). In addition, sensitive genetic markers of cardiac injury/hypertophy, such as atrial and brain natriuretic peptide (Chien et al., 1991), were not induced. Taken together, these results indicate that during normal embryonic development, there is no cardiac myocyte cell–autonomous requirement for the gp130 signaling pathway. Thus, the cardiac defect seen in conventional gp130 knockout embryos represents a secondary effect of gp130 deficiency that may arise as a consequence of the severe hematopoietic defects and subsequent effects on the oxygenation status of the mutant embryo; or the deletion of gp130 in other cell types in the heart may lead to the phenotype in the heart.

Cardiac Function of GP CKO Mice

To investigate whether the cardiac-specific knockout of gp130 would affect in vivo cardiac function, we utilized a panel of in vivo miniaturized physiological technology (Kubalak et al., 1996), including echocardiography (Tanaka et al., 1996) and cardiac catheterization (Palakodeti et al., 1997) in anesthetized mice. As revealed by the echocardiography of multiple littermates, there was no difference in left ventricle (LV) end-diastolic (LVEDD), end-systolic dimension (LVESD), fractional shortening (%FS), septal wall thickness, and posterior wall thickness between GP CKO and CNT mice at baseline (Table 1). Thus global cardiac structure and function was normal. Furthermore, while hemodynamic data revealed

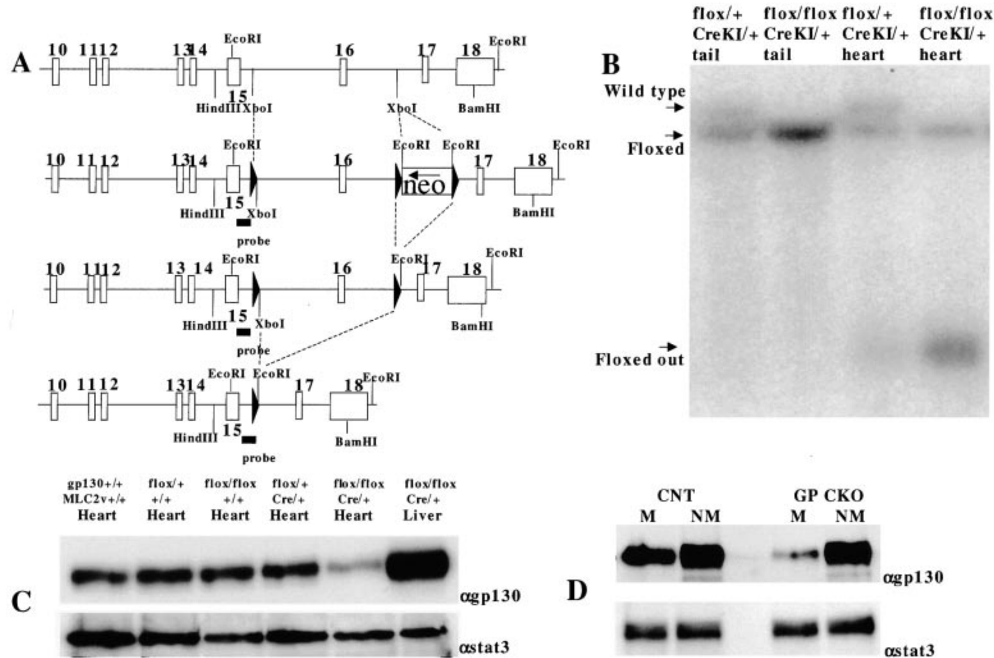

Figure 1. Outline of the Strategy Used to Generate a Ventricular Chamber Restricted Mutation in the *gp130* Gene

(A) Schematic representation of exon structures in the part of the *gp130* gene encoding the transmembrane domain (exon 16). Targeting vector and mutant *gp130* gene are shown. The closed box (probe) corresponds to the sequence used for Southern hybridizaion in (B). Exon numbers are indicated.

(B) Southern genotype analysis. The Southern blot, carrying adult mouse tail and ventricle DNA digested with EcoRI, hybridized with a probe that discriminates between the floxed, floxed out, and wild-type allele. The genotypes for the samples are *gp130flox/+, MLC2vCreKI/+*, tail (lane 1), *gp130flox/flox, MLC2vCreKI/+*, tail (lane 2), *gp130flox/+, MLC2vCreKI/+*, heart (lane 3), and *gp130flox/flox, MLC2vCreKI/+*, heart (lane 4). The position of the bands representing the *gp130*$^{+}$ (wild-type), *gp130*^{flox} (floxed allele), and *gp130*^{delta} (floxed-out allele) alleles are shown.

(C) Expression of gp130 protein and STAT3 were determined by protein immunoblot analysis of myocardial extracts from *gp130+/+, MLC2v+/+*, heart (line 1), *gp130flox/+, MLC2v+/+*, heart (line 2), *gp130flox/flox, MLC2v+/+*, heart (line 3), *gp130flox/+, MLC2vKICre/+*, heart (line 4), *gp130flox/flox, MLC2vKICre/+*, heart (line 5), and *gp130flox/flox, MLC2vKICre/+*, liver (line 6).

(D) Expression of both gp130 and STAT3 proteins were determined by immunoblot analysis of myocardial enriched extracts (M) and nonmyocyte extracts (NM) from control (CNT) and GP CKO hearts. Data are representative of two independent experiments with nearly identical results.

that LV contractility (assessed by LV dp/dtmax) and diastolic function (assessed by LV dp/dtmin) at baseline in GP CKO tended toward a decrease compared to that of the CNT mice, this decrease was not significant as assessed by one-way ANOVA statistical analysis and is within the margin of error noted for other naturally

Table 1. Analysis of In Vivo Cardiac Size and Function by Echocardiography in GP CKO at Basal Level and 2 Days after Transaortic Constriction (TAC)

	CNT Basal (n = 6)	CNT after TAC (n = 6)	GP CKO Basal (n = 7)	GP CKO after TAC (n = 7)
LVEDD (mm)	3.57 ± 0.23	3.37 ± 0.61	3.31 ± 0.36	3.76 ± 0.26
LVESD (mm)	2.31 ± 0.18	2.28 ± 0.48	2.08 ± 0.39	2.91 ± 0.48[c]
FS (%)	35.2 ± 3.02	32.58 ± 3.18	35.25 ± 3.02	22.67 ± 6.35[a,b]
SEPth (mm)	0.69 ± 0.08	0.76 ± 0.16	0.6 ± 0.11	0.69 ± 0.1
PWth (mm)	0.67 ± 0.03	0.71 ± 0.16	0.67 ± 0.13	0.65 ± 0.06
HR (beat/min)	504 ± 77	440 ± 88	417 ± 63	473 ± 127
Mean Vcf (circ/s)	5.9 ± 1.27	5.92 ± 0.87	7.31 ± 1.38	3.83 ± 1.68[a]
BW (g)	25.5 ± 4.2	23.5 ± 4.4	24.7 ± 4.8	21.3 ± 4.8
Mean age at echo (weeks)	7.5 ± 0.84	7.5 ± 0.84	7.71 ± 0.49	7.71 ± 0.49

LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; HR, heart rate; FS, percent fractional shortening calculated as (LVEDD-LVESD)/LVEDD × 100; SEPth, septal wall thickness; PWth, posterior wall thickness; mean Vcf, mean velocity of circumferential fiber shortening; BW, body weight.

[a] $p < 0.001$, GP CKO after TAC versus GP CKO basal and CNT basal.

[b] $p < 0.01$, GP CKO after TAC versus CNT after TAC.

[c] $p < 0.01$, GP CKO after TAC versus GP CKO basal.

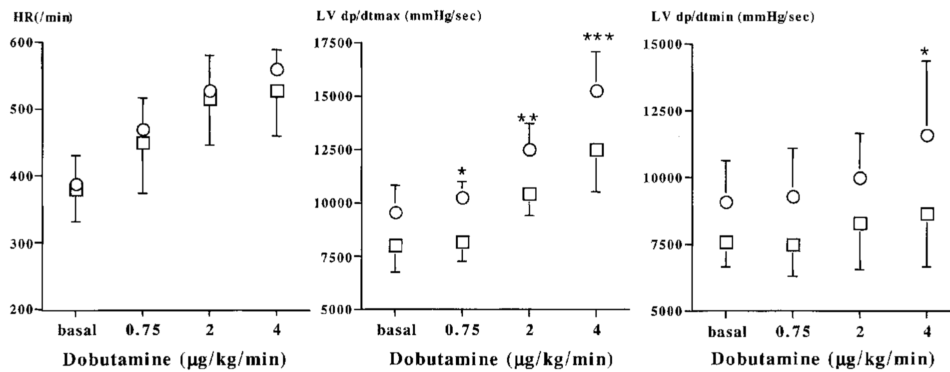

Figure 2. Hemodynamic Data in GP CKO

Retrograde cardiac catheterization at the left ventricle via the carotid artery was performed in intact anesthetized mice using a 1.4 Fr. high-fidelity micromanometer. Parameters are shown at baseline and after progressive infusion of dobutamine in GP CKO (squares; n = 8) and CNT (circles; n = 8) mice. HR, heart rate; LV dp/dtmax, maximal first derivative of LV pressure; LV dp/dtmin, minimal first derivative of LV pressure; *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$, GP CKO versus CNT.

occurring and mutant mice without overt cardiac dysfunction. A normal response to dobutamine was also observed, again consistent with preserved cardiac function, as suggested by the normal echocardiogram (Figure 2). Finally, the GP CKO mice did not display any gross hypertrophy or heart failure phenotype up to six months of age. Taken together, these findings indicated that the GP CKO mice display global cardiac structure and function within normal limits and that the partial loss of gp130 does not by itself lead to cardiac hypertrophy or a heart failure phenotype.

A gp130-Dependent Pathway Is Required for Adaptive Compensatory Hypertrophy during Biomechanical Stress of Pressure Overload

To determine whether gp130 signaling is activated during the biomechanical stress response, we utilized a microsurgical approach to induce cardiac hypertrophy in vivo by pressure overload following transverse aortic constriction (TAC) (Rockman et al., 1991). Subsequently, we performed Western blotting analysis to quantitate the extent of tyrosine phosphorylation of STAT3, a downstream molecule of gp130 activation. At various time points after TAC, the left ventricles were excised from GP CKO and CNT mice. In the left ventricle of CNT mice, STAT3 was phosphorylated within 48 hr following TAC, which was maintained for the entire 7-day period. However, little or no phosphorylation of STAT3 was observed in GP CKO mice following TAC (Figure 3). Thus, the activation of STAT3, a downstream event in the gp130 pathway, occurs early following delivery of the biomechanical stimulus of pressure overload. GP CKO mice display a loss of this STAT3 activation, demonstrating the functional effect of the gp130 conditional knockout. Thus, biomechanical stress induced the activation of gp130 and the downstream STAT3 pathway in cardiac myocytes in the in vivo setting of pressure overload that was lost in GP CKO mice.

The in vivo physiological consequences of the loss of the stress-activated gp130 pathway in GP CKO mice was assessed in vivo using echocardiography 2 days after TAC (Table 1). At this early time point, cardiac contractility was significantly depressed in GP CKO mice ($p < 0.001$), as assessed by fractional shortening and shortening velocity. Although wall thickness and LVEDD were similar to control levels, LVESD significantly increased in GP CKO mice versus control, suggesting the rapid onset of chamber dysfunction and dilation. Strikingly, 7 days after TAC, GP CKO mice displayed a significantly increased mortality as compared with CNT mice with over 90% of the GP CKO mice dying by 7 days after TAC, whereas 70% of the CNT mice remained alive at this time ($p < 0.001$) (Figure 4A). Histological examination of the heart demonstrated ventricular enlargement involving both the left and right cardiac chamber in the gp130-deficient hearts (Figure 4B), providing additional evidence for the rapid onset of a phenotype consistent with dilated cardiomyopathy, as seen in the clinical setting and in analogous mouse model

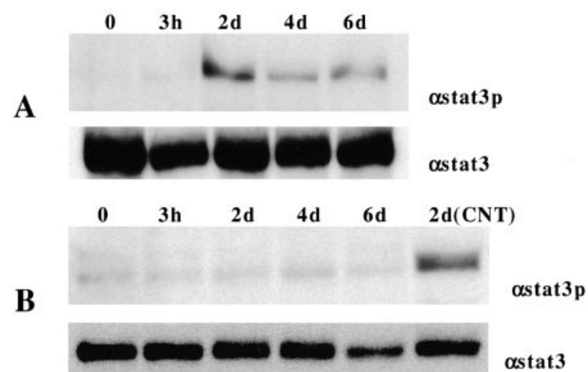

Figure 3. Effect of Pressure Overload on Tyrosine Phosphorylation of STAT3 in CNT (A) and GP CKO (B) Mouse Hearts

Protein extracts from hearts of each group were examined at each point (0, 3 hr, 2 days, 4 days, and 6 days after TAC) were immunoblotted with anti-phospho-STAT3 and STAT3 antibody. Protein extract from the CNT heart 2 days after TAC [2d(CNT)] was used as a positive control in the Western blotting of GP CKO heart extracts (B). Data are representative of two independent experiments with nearly identical results.

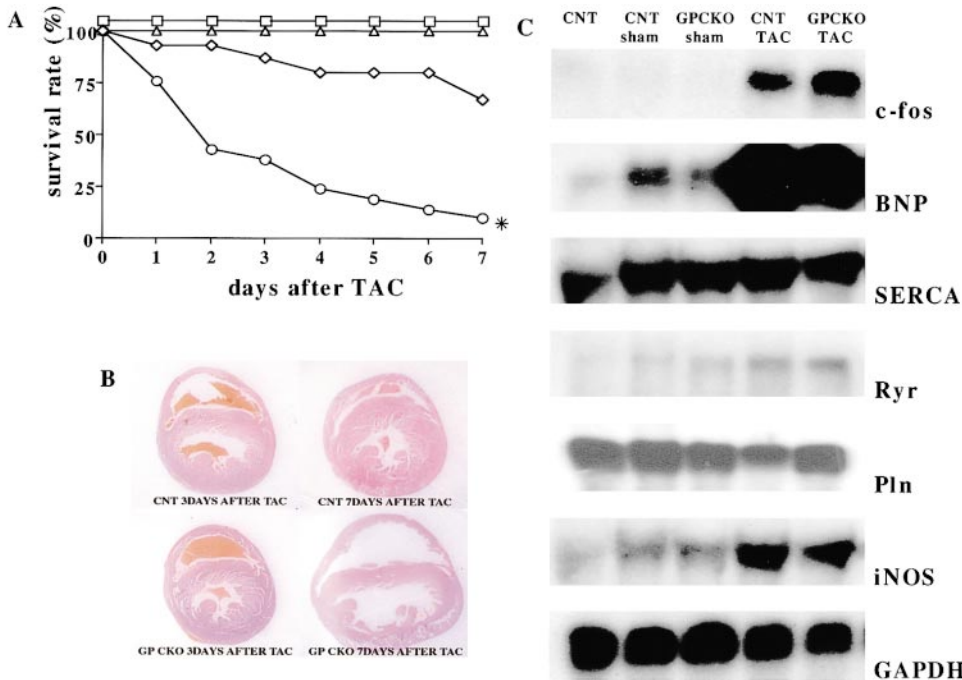

Figure 4. Phenotypic Effects of Biomechanical Stress on GP130 Conditional KO Mice

(A) Survival analysis of gp130 conditional KO mice after TAC. Each group started with 15 (CNT with TAC, diamonds), 21 (GP CKO with TAC, circles), 3 (CNT with sham-operation, squares) and 3 (GP CKO with sham-operation, triangles) mice. Differences in survival rates between the CNT and GP CKO groups after TAC were significant by Peto-Peto-Wilcoxon test (*, $p < 0.001$).

(B) Pathological analysis of the gp130 conditional KO hearts. Histological sections of hearts from 3 days and 7 days after TAC in CNT and GP CKO mice. Heart were fixed in 4% paraformaldehyde and stained with hematoxylin and eosin. Data are representative of three independent experiments with nearly identical results.

(C) Pattern of cardiac gene expression in GP CKO and CNT mice following TAC. Total RNA (10 μg) was isolated from the left ventricle of GP CKO and CNT hearts 3 days after sham-operation or TAC. Northern blot were generated and probed with mouse c-*fos* (1.2 kb fragment) (Van Beveren et al., 1983), mouse *BNP* cDNA (full-length cDNA), rat *SERCA2*α, rabbit *Ryr*, mouse *Pln*, and rat *iNOS*, which was prepared by reverse transcriptase–polymerase chain reaction (Balligand et al., 1994). Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1.2 kbp cDNA) (Adams et al., 1992) was used to control for loading and transfer efficiency. Probes for *SERCA*, *Ryr*, and *Pln* were generous gift from Dr. W. H. Dillmann. Data are representative of three independent experiments with nearly identical results.

systems (Arber et al., 1997). In contrast, control mice developed concentric hypertrophy and maintained a normal chamber volume consistent with an adaptive physiological response (Figure 4B).

gp130-Deficient Hearts Display a Normal Induction of the Embryonic Gene Program during the Biomechanical Stress of Pressure Overload

To further explore the mechanism of the functional cardiac deterioration, the pattern of myocardial gene expression was characterized in pressure-overloaded GP CKO mice. Human heart failure and physiologically relevant experiment models of murine cardiac hypertrophy exhibit increased expression of immediate early genes, embryonic markers, and a panel of genes that encode calcium-handling proteins (Chien et al., 1991). As displayed in Figure 4C, both GP CKO and CNT mice display a greatly enhanced expression of a number of cardiac genes, including c-fos, brain natriuretic factor (BNP), sarcoplasmic reticulum Ca^{2+} ATPase (SERCA), ryanodine receptor (Ryr), phospholamban (Pln), and inducible nitric oxide synthase (iNOS) mRNA following pressure overload. These data indicate that the loss of gp130 in the heart does not affect the induction of the fetal gene program and suggest that the rapid onset of chamber dilation and dysfunction may not be secondary to the loss of the ability to activate a hypertrophic response.

We also considered the possibility that GP CKO mice might have abnormalities in contractile protein expression in the heart. However, no significant abnormalities in contractile protein expression were detected by SDS-PAGE analysis of ventricular protein derived from the GP CKO versus control animals (data not shown).

The Loss of the Stress-Activated gp130 Pathway Leads to Massive Cardiac Myocyte Apoptosis following the Biomechanical Stress of Pressure Overload

As noted earlier, gp130-dependent agonists such as CT-1, are potent myocyte survival factors in vitro (Sheng et al., 1996, 1997), but their in vivo function remains unclear (Wollert and Chien, 1997). Therefore, it became of interest to determine if the rapid onset of cardiac dysfunction observed in the GP CKO mice following TAC was associated with an increase in stress-activated myocyte apoptosis in these animals. Three independent assays were used to directly compare apoptosis in GP CKO and CNT hearts 7 days after TAC. As shown in

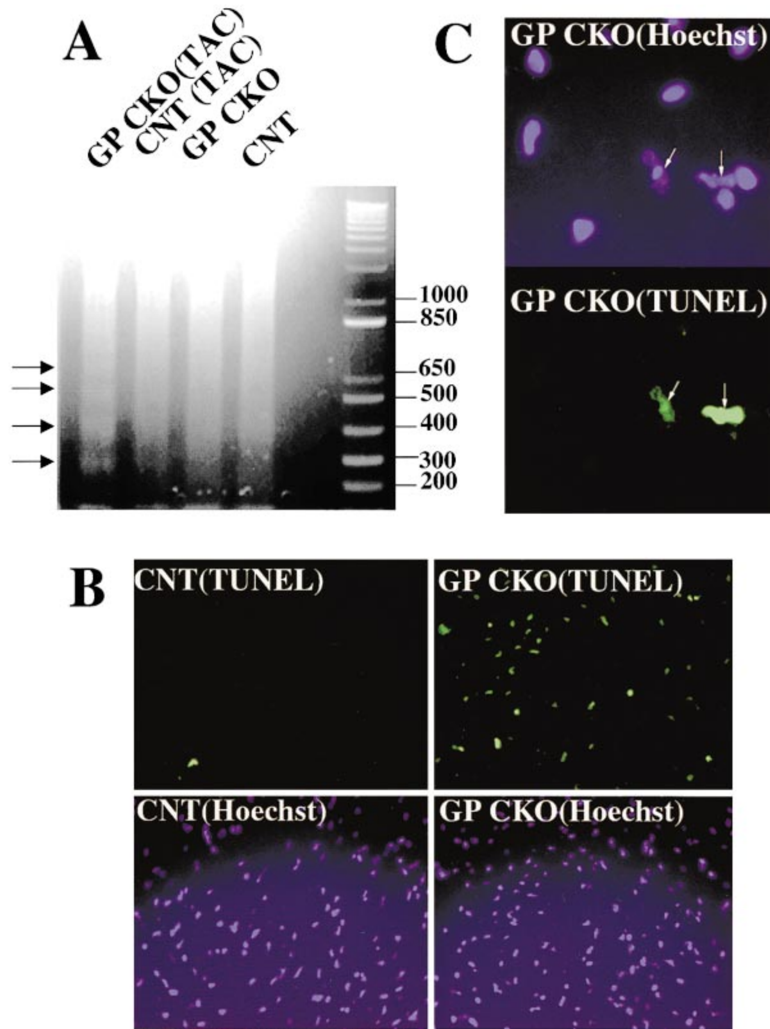

Figure 5. DNA Strand Breaks in Cardiac Myocyte Nuclei of GP CKO after TAC

(A) DNA laddering of GP CKO cardiac DNA after TAC. (B) Low power (200×) and (C) high power (1000×) images showing nuclear DNA labeling visualized by fluorescence (green) and counterstained with Hoechst dye (blue). Apoptotic nuclei appear green by TUNEL staining and demonstrate varying degrees of condensed nuclear chromatin (arrow).

Figure 5A, a DNA laddering assay revealed evidence of an increase in apoptosis in the GP CKO hearts after TAC. In a second series of experiments, we used a TUNEL assay to compare the number of apoptotic cardiac myocytes in GP CKO and CNT mice after TAC. In agreement with the DNA laddering assay, there was significant increase in apoptotic cells in GP CKO hearts (Figure 5B), with apoptotic indices of 34% $\pm$ 11% in GP CKO TAC versus 3% $\pm$ 0.5% in CNT mice following TAC ($n = 4$ each, $p < 0.05$). Cells with apoptotic nuclei were also identified as cardiac myocytes by α-sarcomeric actin stain (data not shown) and bisbenzimide (Hoechst dye 22358) staining. TUNEL-stained nuclei demonstrated varying degrees of condensed nuclear chromatin (Figure 5C), again consistent with a massive induction of cardiac apoptosis that involved at least one-third of the ventricular cardiomyocyte population.

Discussion

Ventricular-Restricted Targeting of the *gp130* Gene Reveals Non–Cardiac Myocyte Cell–Autonomous Requirement during Normal Cardiac Growth and Development

The IL-6 family of cytokines have pleiotropic effects on multiple organ systems, including hematopoiesis, the immune system, bone metabolism, neuronal structure and function, and intermediary metabolism (Taga and Kishimoto, 1997). Mice that harbor a complete *gp130* gene ablation display multiple defects, including hematopoietic and cardiac defects that ultimately lead to embryonic lethality (Yoshida et al., 1996). In addition, the generation of mice that harbor a postnatal deficiency of gp130 in multiple tissues display a number of defects, including neurological, cardiac, hematopoietic, immunological, hepatic, and pulmonary defects (Betz et al., 1998). To a large extent, these widespread effects reflect their shared use of common gp130 signaling pathways to orchestrate downstream responses in diverse differentiated cell types (Kishimoto et al., 1994). Dissecting out which of these phenotypes reflect primary versus secondary requirements is now possible by controlling the spatial onset of the targeting event (Rajewsky et al., 1996).

Utilizing a knock-in strategy of Cre recombinase into the endogenous MLC2v gene locus (Chen et al., 1998a, 1998b), we engineered a ventricular restricted knockout of the *gp130* gene at the earliest stages of ventricular development. We have now provided clear evidence that the cardiac developmental defects and associated embryonic lethality do not reflect a requirement of gp130 pathways in cardiac myocytes per se. The most likely

explanation is that the cardiac defects and thinning of the ventricular chamber wall that resembles a number of other cardiac embryonic lethal mutations, arises secondary to the hematopoietic defect and associated oxygen deprivation. Interestingly, the gp130 pathway also does not appear to be required for normal heart growth in the postnatal setting.

This distinction between a primary and secondary requirement for a discrete signaling pathway has been shown to be particularly important with respect to the growing number of mice that harbor defects in cardiac chamber morphogenesis and that also die as a result of embryonic heart failure (for a review, see Fishman and Chien, 1997). For example, previous studies have shown that the retinoid receptor RXRα is responsible for the vitamin A signaling pathways that are required to maintain normal cardiac morphogenesis (Kastner et al., 1994; Sucov et al., 1994; Dyson et al., 1995; Gruber et al., 1998). Mice that harbor a complete ablation of the *RXRα* gene display all of the features of vitamin A deficiency, including defects in neural crest–related defects in the outflow tract, atrioventricular cushion defects (Gruber et al., 1996), and severe ventricular muscle defects that result in marked thinning of the ventricular chamber wall and secondary embryonic heart failure with associated in vivo cardiac dysfunction (Ruiz-Lozano et al., 1998). However, recent studies based on mice that harbor a ventricular restricted knockout of the *RXRα* gene have indicated that all of these defects do not reflect a requirement for RXRα in ventricular muscle cells per se, but rather reflect a requirement from another cardiac cellular compartment, most likely via a paracrine pathway (Chen et al., 1998b). Thus, the cardiac muscle defects that are related to embryonic lethality do not reflect an intrinsic requirement for RXRα in heart muscle, and the muscle defect is related to a secondary effect. Taken together with the results of the current study, the "thin wall" myocardial defect seen in a wide number of gene-targeted embryos may likely indicate a secondary as opposed to a primary defect of the targeted gene.

Biomechanical Stress Activates a gp130 Cardiomyocyte Survival Pathway

Biomechanical stress during chronic increases in blood pressure and circulatory volume is a major determinant of cardiac function and the transition to heart muscle failure. In response to mechanical overload, the heart activates an adaptive physiological response of cardiac muscle hypertrophy, that acts to increase muscle mass and to maintain normal cardiac function (Chien et al., 1998). As a result, the onset of chamber dilation, cardiomyopathy, and overt heart failure are usually only seen after months or years of chronic exposure to the initiating bioimechanical stimulus (e.g., high blood pressure, valvular disease, and postmyocardial infarction). The identification of the precise signaling pathways that mediate this stress-activated response is one of the most important questions in cardiovascular biology and medicine (Chien, 1993). Of particular importance would be the identification of genetic modifiers of the response that act to promote or suppress the onset of the heart failure phenotype in the setting of exposure to biomechanical stress. Such pathways could become important targets for the development of new therapeutic strategies for heart failure.

In this regard, the present study provides clear evidence that gp130 is part of an essential stress-activated myocyte survival pathway. While there is no apparent requirement for gp130 in the normal physiological response of the heart, gp130 pathways are rapidly activated in response to the mechanical stress of pressure overload. Following thoracic aortic constriction, there is a marked increase in cardiac mortality, such that almost none of the ventricular-restricted gp130 knockout mice survive past 7 days following imposition of a 35 mm pressure transaortic gradient. Within 3–4 days of aortic banding, the gp130 ventricular restricted knockout mice display a rapid onset of dilated cardiomyopathy, which is accompanied by massive myocyte apoptosis, involving over 30% of the ventricular myocytes. Taken together, these studies provide evidence that gp130 mediates a myocyte survival pathway that acts to block the onset of myocyte apoptosis during pressure overload. In this manner, mechanical stimuli may activate not only pathways that promote myocyte apoptosis, but also may concomitantly activate myocyte survival pathways.

Recently, our laboratory has obtained additional evidence in vitro that cardiac myocyte apoptosis and hypertrophy may be activated by common signaling pathways. This work emanates from previous studies, which eventually resulted in the identification and cloning of CT-1 (Pennica et al., 1995), a novel cardiac cytokine that was cloned from an embryonic stem cell in vitro cardiogenesis model system (Miller-Hance et al., 1993). Utilizing expression cloning technology, CT-1 was identified as a novel agent to promote cardiac myocyte hypertrophy in a high-throughput in vitro neonatal rat myocardial cell assay system (Pennica et al., 1995). The structure of CT-1 indicated that it was a member of the IL-6 family that operates through the common signal-transducing membrane receptor gp130 (for review, see Wollert and Chien, 1997). Subsequent studies from our laboratory documented that a gp130-LIF receptor β heterodimer constituted the CT-1 receptor on the surface of cardiac myocytes (Wollert et al., 1996), and that subsequent activation of JAK and STAT3 were responsible for the induction of a hypertrophic response and activation of a panel of embryonic genes (Sheng et al., 1996, 1997). In subsequent studies, we documented that CT-1 could promote cardiac myocyte survival of both embryonic and neonatal cardiac muscle cells (Sheng et al, 1996, 1997). These studies support the concept that the genetic ablation of the gp130 receptor and the subsequent crippling of myocyte survival patterns in vivo are due to a direct effect of gp130-dependent ligands on ventricular cardiomyocytes. Taken together with the results of the present study, we conclude that myocyte survival pathways and the corresponding blockade of myocyte apoptosis may be critical in the onset of compensatory cardiac hypertrophy versus the transition to heart failure. Based on the current and previous studies, a working model can be proposed whereby biomechanical stress induces hypertrophic signals (Ras, Gq, p38β, others) (Thorburn et al., 1993; LaMorte et al., 1994; Gottshall et al., 1997; Wang et al., 1998a) and apoptotic signals (Gq, p38α, others) (Adams et al., 1998; Wang et al., 1998a, 1998b) that represent two distinct phenotypic outcomes. At the same time, biomechanical signals lead to the release of gp130 ligands that activate a myocyte survival pathway, shifting the balance toward adaptive

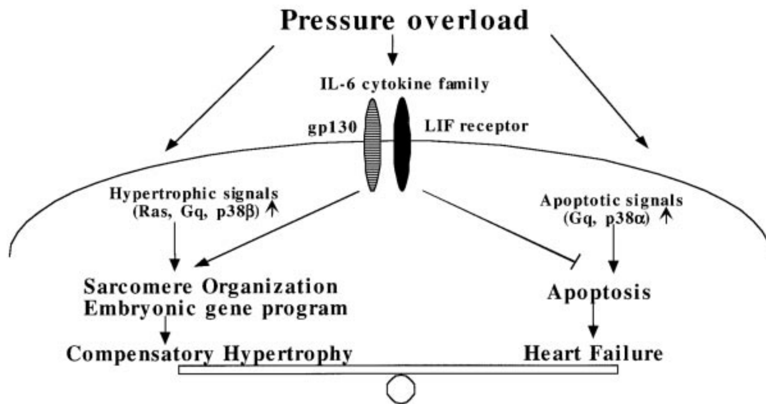

Figure 6. The Proposed Role of gp130 Signaling Pathways in the Transition between Cardiac Hypertrophy and Heart Failure in Response to Biomechanical Stress

Biomechanical stress activates multiple parallel and converging signals for hypertrophy and apoptosis that represent two distinct phenotypic outcomes (for a review, see Chien et al., 1998). At the same time, biomechanical stress also leads to the induction of gp130-dependent ligands, resulting in the activation of downstream gp130 pathways that act to block the actions of proapoptotic pathways. In the absence of gp130, the cardiac myocyte response to biomechanical stress is shifted toward cell death, resulting in the loss of functional myocytes and the onset of heart failure. Thus, the phenotypic outcome of biomechanical stress is dependent on the balance between the contradictory signal transduction pathways.

hypertrophy. In this regard, semiquantitative RT-PCR documented that LIF was significantly increased ($p < 0.05$) in the heart 2 days after TAC (data not shown). In the absence of the gp130 signaling pathway in the heart, there is an acceleration of this transition to heart failure that most likely occurs through an unopposed effect of apoptotic pathways in the setting of chronic mechanical stress (Figure 6).

The Loss of Myocyte Survival Is Critical in the Transition to Dilated Cardiomyopathy and Heart Failure

Recent studies have documented that apoptosis can occur in the setting of cardiac hypertrophy and failure in both experimental model systems and in the setting of human congestive heart failure (for review, see Haunstetter and Izumo, 1998). However, the extent to which apoptosis plays a critical role in the transition between compensatory hypertrophy and overt cardiac failure has been an open question, due to the difficulty of scoring for myocyte apoptosis, which can be an infrequent event even in the setting of cardiac hypertrophy and heart failure. In addition, scoring for the event at an individual cell level has also been problematic, as well as a means to specifically quantitate the rate and extent of apoptosis in cardiac myocytes per se, as opposed to infiltrating cardiac muscle cells or nonmyocytes that comprise the majority of cell types within the heart.

The results of the current study provide the strongest evidence to date that myocyte apoptosis is a critical event in the transition to heart failure. Thus, the inhibition of cardiac myocytes apoptosis is now clearly a valid target for the development of new therapeutic agents for cardiac heart failure. As noted earlier, our working model suggests that, under normal circumstances, the myocyte survival pathways can effectively blunt the onset of myocyte apoptosis in response to biomechanical stress, leading to the induction of compensatory hypertrophy (see Figure 6). However, at a later point in time, this balance has tipped, such that there is a transition to dilated cardiomyopathy. Accordingly, identifying the downstream pathways by which gp130-dependent ligands can promote cardiac myocyte survival becomes of critical interest. Given the importance of STAT3 in in vitro function of CT-1, the question arises as to whether all of the gp130-dependent survival effect is mediated via STAT3 pathways. The possibility exists that modification of gp130-mediated myocyte survival pathways represents a novel therapeutic strategy for promoting and maintaining cardiac myocyte function in response to acute mechanical and/or other stress-induced stimuli (e.g., hypoxia, adriamycin, etc.). The availability of this engineered mouse model should prove useful in the identification of new downstream targets for promoting in vivo cardiac myocyte survival and preventing the transition to heart failure during biomechanical stress.

Experimental Procedures

In Vivo Physiological Assays

Breeding and identification of crosses between *MLC2vCre* and *gp130* floxed allele mice was performed as previously described (Chen et al., 1998b). Transthoracic echocardiography (Tanaka et. al., 1996), hemodynamic evaluation (Arber et al., 1997), and transverse aortic constriction (Rockman et al., 1991) was performed as previously described.

Isolation of Cardiac Myocyte

Adult ventricular cells were isolated by retrograde perfusion with collagenase and two sequential 6% BSA gradients by a modification (Chen et al., 1998b) previously described (Wolska and Solaro, 1996).

Western and Northern Blot Analysis

Western blot analysis was performed utilizing rabbit polyclonal antibodies to gp130, STAT3 (Santa Cruz), and phospho-specific STAT3 (New England Biolabs Inc.). Horseradish peroxidase-conjugated anti-rabbit immunoglobulin G was used for detection (Santa Cruz). Northern blotting was performed as previously described (Rockman et al., 1991).

Apoptosis Assay

For internucleosomal DNA cleavage assays, DNA preparation and agarose electrophoresis were performed essentially as described (Teiger et al., 1996; Olivetti et al., 1997; Haunstetter and Izumo, 1998). For the terminal deoxytransferase assay (TUNEL), we used the Apoptag kit (Oncor) for in situ apoptosis detection.

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