Diminished GATA4 Protein Levels Contribute to Hyperglycemia-induced Cardiomyocyte Injury*

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Hyperglycemia is an independent risk factor for diabetic heart failure. However, the mechanisms that mediate hyperglycemia-induced cardiac damage remain poorly understood. The transcription factor GATA4 is essential for cardiac homeostasis, and its protein levels are dramatically reduced in the heart in response to diverse pathologic stresses. In this study, we investigated if hyperglycemia affects GATA4 expression in cardiomyocytes and if enhancing GATA4 signaling could attenuate hyperglycemia-induced cardiomyocyte injury. In cultured rat cardiomyocytes, high glucose (HG, 25 or 40 mM) markedly reduced GATA4 protein levels as compared with normal glucose (NG, 5.5 mM). Equal amount of mannitol did not affect GATA4 protein expression (NG, 100 ± 12%; mannitol, 97 ± 8%, versus HG, 43 ± 16%, p < 0.05). The GATA4 mRNA content, either steady-state or polysome-associated, remained unchanged. HG-induced GATA4 reduction was reversed by MG262, a specific proteasome inhibitor. HG did not activate the ubiquitin proteasome system (UPS) in cardiomyocytes as indicated by a UPS reporter, nor did it increase the peptidase activities or protein expression of the proteasomal subunits. However, the mRNA levels of ubiquitin-protein isopeptide ligase (E3) carboxyl terminus of Hsp70-interacting protein (CHIP) were markedly increased in HG-treated cardiomyocytes. CHIP overexpression promoted GATA4 protein degradation, whereas small interfering RNA-mediated CHIP knockdown prevented HG-induced GATA4 depletion. Moreover, overexpression of GATA4 blocked HG-induced cardiomyocyte death. Also, GATA4 protein levels were diminished in the hearts of streptozotocin and db/db diabetic mice (44 ± 7% and 67 ± 13% of control, p < 0.05), which correlated with increased CHIP mRNA abundance. In summary, increased GATA4 protein degradation may be an important mechanism that contributes to hyperglycemic cardiotoxicity.

Diabetes is a major risk factor for the development of various cardiovascular diseases, including atherosclerosis, hypertension, and diabetic cardiomyopathy, which collectively constitute the leading causes of mortality from diabetes. Diabetic cardiomyopathy, independent of vascular pathology, is now recognized as an important causative factor for the heightened risk of heart failure and mortality in diabetic patients (1, 2). The characteristic metabolic abnormalities of diabetes include hyperlipidemia, hyperinsulinemia (type 2 diabetes), and hyperglycemia. The development of diabetic cardiomyopathy correlates with the duration and severity of hyperglycemia. Hyperglycemia induces cardiac damage through a number of biochemical mechanisms, including the formation of advanced glycation end products (3), altered calcium homeostasis (4), increased renin-angiotensin system (5), and protein kinase C activation (6). Ultimately, it is thought that hyperglycemia increases oxidative stress inducing cardiomyocyte death leading to heart failure in animals (7, 8) and humans (9) with diabetes. In fact, high concentrations of glucose induce the generation of reactive oxygen species (ROS)4 and cell death in cultured cardiomyocytes (7, 10–13). The importance of oxidative stress in diabetic cardiomyopathy is underscored by the ability of various antioxidants to prevent diabetic cardiac damage in animal studies (10, 14, 15). However, antioxidants are not efficacious in human diabetic cardiomyopathy (16, 17), suggesting that mechanisms other than ROS generation might be involved.

GATA4 is a cardiac-enriched zinc finger–containing transcription factor that belongs to the GATA superfamily, which is composed of six members. GATA4 regulates the expression of various cardiac genes ranging from contractile proteins to peptide hormones and transcription factors (18). Accordingly, GATA4 is essential for several developmental processes (18) and a number of adaptive responses in the heart, including myocyte survival and cardiac hypertrophy (19, 20). As a survival factor, GATA4 contributes to cytoprotection in cardiomyocytes induced by endothelin-1 (21) and ischemic preconditioning (22, 23). GATA-4 is markedly reduced in endotoxin or

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The abbreviations used are: ROS, reactive oxygen species; STZ, streptozocin; NRVC, neonatal rat ventricular cardiomyocytes; NG, normal glucose; HG, high glucose; RT, reverse transcriptase; UPS, ubiquitin proteasome system; CHIP, carboxyl terminus of Hsp70-interacting protein; MuRF1, muscle ring finger protein-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; E3, ubiquitin-protein isopeptide ligase; TUNEL, terminal deoxynucleotidyltransferase-mediated nick-end labeling; DMEM, Dulbecco’s modified Eagle’s medium; siRNA, small interfering RNA; PI, propidium iodide.

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infarction-induced failing hearts, and treatments that improve cardiac function also restore GATA4 levels (24, 25). Moreover, the anticancer drug doxorubicin depletes GATA4 protein levels and induces apoptosis in cardiomyocytes, which is prevented by overexpression of GATA4 (26). In addition, genetic inactivation of GATA4 in the heart induces apoptosis and impairs cardiac function (20). Collectively, these results indicate that GATA4 is an essential myocardial survival factor that can protect against cardiac injury elicited by a wide variety of pathological conditions. However, it remains unclear if GATA4 plays any role in hyperglycemia-induced cardiomyocyte injury and diabetic cardiomyopathy.

In this study, we examined the effect of hyperglycemia on the survival factor GATA4 in cultured cardiomyocytes to identify novel mechanisms of diabetic cardiomyopathy. Our results demonstrate that high glucose induces GATA4 depletion in cardiomyocytes by increased degradation of GATA4 protein through the ubiquitin proteasome system (UPS). This is likely mediated by high glucose-induced expression of the E3-ubiquitin ligase CHIP. We also found that overexpression of GATA4 prevents hyperglycemia-induced GATA4 depletion and myocyte death. In addition, in mouse models of both type 1 and type 2 diabetes, GATA4 levels are decreased, and CHIP levels are increased. In summary, our findings identify a potentially novel mechanism to explain hyperglycemic cardiotoxicity and diabetic cardiomyopathy.

**EXPERIMENTAL PROCEDURES**

**Neonatal Rat Ventricular Cardiomyocyte (NRVC) Culture and High Glucose Treatment**—NRVCs were isolated from 0- to 2-day-old Harlan Sprague-Dawley rats using a kit from Worthington as described previously (27). For hyperglycemia studies, the NRVCs were cultured in Dulbecco’s modified essential medium (DMEM containing 1% of penicillin/streptomycin; Invitrogen) with 5.5, 25, or 40 mmol/liter of glucose. To control for changes in medium osmolarity caused by increased glucose concentration, NRVCs were cultured in medium containing equivalent amounts of mannitol.

**Western Blot Analysis**—Protein extracts were prepared, and the Western blot analysis was performed as described previously (27). Primary antibodies were obtained from the following vendors: GATA4, Bcl2, NXX2.5, Bax, and GFP from Santa Cruz Biotechnology; α-actinin from Sigma; 20 S proteasome subunits α2-7, β1, and β2 and 19 S proteasome subunits RP2 and RPT5 from Biomol; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from Research Diagnostics Inc. (Concord, MA).

**Semi-quantitative RT-PCR**—RT-PCR was carried out using the TaqMan reverse transcription reagents (Applied Biosystems, Foster City, CA) as described (27). Two μl of the cDNA was used for PCR amplification. The gene-specific primers for GATA4, Bcl2, Bax, and GAPDH were described previously (27). The primer sequences for other genes are as follows: atrogin1 forward, 5’-ACTGGACTTCTCGACTGCCAT, and reverse, 5’-CTCCATCGGATACACCCACAT; MuRF1 forward, 5’-AACACTCTCAGGGAAGAAGGAA, and reverse, 5’-TGCAAGGTGTGGGCGCT; CHIP forward, 5’-AGGCAGAGGGAAGAAAGA, and reverse, 5’-TGGCAATGGCCCTATCATATA; 18 S ribosomal RNA forward, 5’-GTCCCCAACTTGTAGAG, and reverse, 5’-CACCTACGGGAAACCTTGTAC.

**Analysis of Polysome-associated mRNA**—The polysome-associated GATA4 mRNA was analyzed according to published procedures (28). NRVCs were cultured in DMEM containing 5.5 or 40 mmol/liter glucose for 3 days. Cells were washed with ice-cold phosphate-buffered saline and lysed in polysome lysis buffer containing 100 mmol/liter KCl, 10 mmol/liter MgCl2, 1 mmol/liter dithiothreitol, 0.5% Nonidet P-40, 100 μg/ml cycloheximide, 10 units/ml of RNase inhibitor, and 10 mmol/liter Tris-HCl, pH 7.4. The amounts of RNA containing cytoplasmic lysates equivalent to 20 A at 260 nm were loaded onto a linear sucrose gradient in polysome lysis buffer. Gradients were centrifuged at 256,000 × g for 100 min at 4 °C. From the top of gradients, 35 × 300-μl fractions were sequentially removed and their absorbances at 260 nm were measured and plotted. The polysome-bound RNAs were extracted from each sample pooled from three continuous fractions and used for RT-PCR.

**Gene Silencing with siRNA**—GATA4 siRNA was designed based on sequence specific for both mouse and rat GATA4 cdNA (5’-GAGGGGATTTCGACAACAGA-3’) and purchased from Ambion (Austin, TX). Three different pre-designed siRNAs targeting rat CHIP mRNA (28–30) and human GAPDH siRNA were also obtained from Ambion. siRNA transfection with a final concentration of 50 nmol/liter was performed using Lipofectamine RNAiMAX according to the manufacturer’s instructions (Invitrogen). Briefly, 0.7 × 10⁶ NRVCs were transfected in 3 ml of serum-free and antibiotics-free DMEM containing 500 μl of Opti-MEM (Invitrogen), 6 μl of Lipofectamine RNAiMAX, and 50 nmol/liter of each siRNA. The media were replaced 12 h later with fresh serum-free media containing 5.5 or 40 mmol/liter glucose. The cells were harvested 60 h later for RNA extraction or Western blotting.

**Measurement of Myocyte Death**—Myocyte death was measured by propidium iodide (PI) staining (nonspecific indicator of cell death), TUNEL staining (apoptosis), and DNA laddering (apoptosis). Myocytes were cultured for 72 h with different concentrations of glucose. PI (Roche Applied Science) was added directly to the culture medium, and myocytes were photographed under both phase contrast and fluorescent conditions. For TUNEL staining, myocytes were labeled by terminal deoxynucleotidyltransferase-mediated nick-end labeling (TUNEL) using the In Situ Cell Death Detection kit (Roche Applied Science). Myocytes were counter-stained with 4,6-diamidino-2-phenylindole (DAPI) and Alexa Fluor 488-conjugated phalloidin. For both PI and TUNEL staining at least 200 myocytes were examined from each sample, and each condition was measured in triplicate. For DNA laddering, we used a semi-quantitative PCR-based DNA laddering kit (Maxim Biotech, San Francisco, CA) as described (29).

**Adenoviral Constructs**—Adenoviruses expressing β-galactosidase, GATA4, CHIP, atrogin1 (muscle atrophy F-box), or MuRF1 (muscle ring finger protein-1) were described previously (19, 30–32).

**Measurement of UPS Activity and 20 S Peptidase Activity**—UPS proteolytic function in NRVCs was assayed by an adenovirus encoding a GFPu reporter (33). The chymotrypsin-, caspase-, and trypsin-like activities of 20 S proteasome were
determined by using the synthetic fluorogenic peptide substrates as described previously (33).

**Diabetic Mouse Models**—Type 1 diabetes was induced in mice by injecting 2-month-old FVB mice with a single dose of streptozotocin (STZ) (intraperitoneally, 150 mg/kg body weight in 10 mmol/liter sodium citrate, pH 4.5), a well-established agent that destroys pancreatic beta cells (34). Fasting blood glucose content of 15 mmol/liter or greater was considered diabetic, whereas vehicle-treated mice were used as controls. The heart tissues from type 2 diabetic db/db mice and their control db/+ mice were kindly provided by Dr. Paul Epstein, University of Louisville. The db/db mice are a well-characterized insulin-resistant diabetic model with early onset cardiomyopathy (35). All protocols involving animal use were reviewed and approved by the Internal Animal Care and Use Committee at the University of South Dakota.

**Statistical Analysis**—Data are expressed as mean ± S.E. In all experiments, values were compared by two-tailed Student’s *t* test or one-way analysis of variance, and *p* < 0.01 or 0.05 was considered significant.

**RESULTS**

**High Glucose Diminishes the Cardiac Transcription/Survival Factor GATA4**—Hyperglycemia is an independent risk factor for diabetic heart failure (2). In cultured cardiomyocytes, high glucose induces ROS generation and apoptosis (7, 10–13). In animal models of diabetes, antioxidants attenuate heart failure, suggesting a mechanistic link between hyperglycemia-induced ROS generation, myocyte death, and heart failure (10, 14, 15). However, antioxidants show limited efficacy in preventing diabetic heart failure in humans, indicating other mechanisms might contribute (16, 17).

Diverse pathological insults deplete GATA4 protein levels in the heart, including endotoxin (24), doxorubicin (36), and myocardial infarction (25). To identify alternative mechanisms to explain diabetic heart injury, we examined the effect of high glucose (HG) on expression of the cardiac-specific transcription/survival factor GATA4 in cultured NRVC. HG (25 or 40 mmol/liter) markedly reduced GATA4 protein levels in NRVCs as compared with normal glucose (5.5 mmol/liter, NG) (Fig. 1A). Increased culture medium osmolarity did not explain the reduction in GATA4 induced by HG in NRVCs, because normalizing osmolarity with mannitol (5.5 mmol/liter glucose +
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A

B

C

D

34.5 mmol/liter mannitol) did not alter GATA4 protein levels (Fig. 1, B and C; NG 100 ± 12%, mannitol 97 ± 8% versus HG 43 ± 16%, n = 4, p < 0.05). Conversely, HG did not alter GATA4 mRNA levels in NRVCs as determined by RT-PCR (lower panel, Fig. 1B), suggesting a post-transcriptional regulation of GATA4 protein levels by HG. Moreover, HG did not significantly reduce the protein levels of NKK2.5 (Fig. 1B), another cardiac specific transcription factor, suggesting that the HG effect on GATA4 is relatively specific.

To examine post-transcriptional regulation of GATA4 by high glucose, we measured the association of GATA4 mRNA with high molecular weight polysome complexes to assess the rate of GATA4 translation in NRVCs cultured with NG or HG. Cytoplasmic cell lysates were passed through a sucrose gradient to separate polysome-bound mRNA, which was then analyzed by RT-PCR. HG had no effect on the absorption profile of polysomes at 260 nm in NRVCs (Fig. 1D). More importantly, HG had no effect on the relative abundance of GATA4 mRNA bound to each of the polysomal fractions (Fig. 1E), indicating that the levels of polysome-associated GATA4 mRNA were not reduced in HG-treated NRVCs. In summary, these findings demonstrate that HG depleted GATA4 in NRVCs by mechanisms other than reduced GATA4 transcription or translation.

HG-induced GATA4 Reduction Is Mediated by UPS—UPS-mediated protein degradation is a multistep process involving ubiquitination of the target protein followed by proteasome-mediated degradation. Protein ubiquitination is regulated by a cascade of enzymatic reactions involving the ubiquitin-activating enzyme, a ubiquitin-conjugating enzyme, and an E3. Multiple ubiquitin moieties are conjugated to the substrate, which permits recognition and degradation of the substrate by the 26 S proteasome (37). The 26 S proteasome is composed of two 19 S regulatory complexes and a catalytic core 20 S proteasome. The 19 S complex recognizes and removes the ubiquitin moieties from labeled proteins and transfers them to the 20 S proteasome for degradation. The specific peptidase activities residing in the 20 S proteasome are responsible for protein degradation.

To determine whether HG-induced GATA4 depletion is regulated by the UPS, NRVCs were cultured in HG with MG262 (25 nmol/liter), a specific proteasome inhibitor. HG-induced GATA4 depletion was completely reversed by MG262 (Fig. 2). In fact, MG262 increased GATA4 levels in both NG- and HG-treated NRVCs, indicating that UPS-mediated GATA4 degradation is an important regulator of basal GATA4 levels as well (Fig. 2). These results suggest that HG-induced GATA4 depletion was likely mediated by the UPS.

HG Does Not Increase UPS Proteolytic Activity—To examine the role of the UPS in HG-induced GATA4 depletion, we measured the effect of HG on UPS activity by using a previously described GFPu UPS reporter (33). The GFPu protein carries a consensus degradation signal known as degron CL1 that renders the protein sensitive to UPS-mediated degradation. The protein level and the fluorescence intensity of GFPu are inversely related to the UPS proteolytic activity (33). Unexpectedly, HG did not increase UPS-mediated degradation of GFPu in NRVCs, shown by the slightly increased levels of the GFPu protein (Fig. 3A). In addition, we measured trypsin-like, caspase-like, and chymotrypsin-like activities associated with the 20 S proteasome. However, HG inhibited caspase-like activity and had no effect on trypsin- and chymotrypsin-like activity in NRVCs (Fig. 3B). The 20 S and 19 S proteasomes each contain multiple subunits with distinct structural and functional roles (37). Once again however, HG had no effect on protein levels of the 20 S (α2-7, β1, and β2) or 19 S (RP2 and RPT5) subunits in NRVCs (Fig. 3C). Mannitol mimicked the effect of HG and slightly inhibited the UPS activity (Fig. 3A, slightly increased GFPu levels), significantly reduced caspase-like activity (Fig. 3B), and had no effect on 20 S or 19 S protein levels (Fig. 3C). In summary, HG induced-GATA4 depletion was not associated with a general activation of the UPS, and UPS proteolytic activity does not appear to be rate-limiting in GATA4 protein degradation. Alternatively, increased ubiquitination per se might enhance protein degradation simply by directing more modified GATA4 protein to the 26 S proteasome even if the proteasome activity is moderately reduced.

Increased Expression of the E3 Ubiquitin Ligase CHIP Mediates HG-induced GATA4 Depletion—Ubiquitin E3 ligases determine the relative specificity of substrates that undergo proteasome-mediated degradation (37). Therefore, HG might activate a specific E3 ligase that facilitates GATA4 ubiquitination and degradation even if proteasome activity is moderately inhibited. We screened several E3 ligases that are expressed in cardiomyocytes, including atrogin1, MuRF1, and CHIP. HG...
dramatically induced mRNA expression of CHIP but not atrogin1 or MuRF1 in cultured NRVCs as determined by RT-PCR (Fig. 4A). Importantly, adenovirus-mediated gene transfer of CHIP, but not atrogin1 or MuRF1, depleted GATA4 protein levels in NRVCs (Fig. 4B), whereas CHIP gene silencing by three different siRNAs dramatically reduced CHIP mRNA levels (Fig. 4C), which prevented high glucose-induced GATA4 depletion (Fig. 4D). These results strongly support the hypothesis that CHIP may be the E3 ligase that is responsible for hyperglycemia-induced GATA4 degradation.

_Overexpression of GATA4 Prevents HG-induced GATA4 Degradation and Myocyte Death—_High glucose results in ROS generation and cardiomyocyte apoptosis (7). Our results demonstrate that HG also depletes GATA4, possibly increasing susceptibility to HG-induced cell death and suggesting a novel mechanism for hyperglycemic cardiotoxicity. Therefore, to test the hypothesis that GATA4 overexpression could rescue myocytes from HG-induced GATA4 depletion and cell death, we infected NRVCs with adenovirus encoding GATA4 (AdGATA4) or β-galactosidase (Adβgal), cultured them in the presence of NG or HG, and measured GATA4 levels and cell death. Overexpression of GATA4 prevented GATA4 depletion and up-regulated Bcl-2 expression in NRVCs (Fig. 5A). Correspondingly, overexpression of GATA4 prevented HG-induced cell death as indicated by reduced PI-positive cells (Fig. 5, B and C), TUNEL-positive cells (Fig. 6, A and B), and DNA laddering (Fig. 6C). Increased GATA4 also reduced HG-induced ROS generation measured by CM-H2DCFDA fluorescent intensity (data not shown). In summary, GATA4 overexpression is sufficient to prevent HG-induced GATA4 depletion and attenuate hyperglycemia-induced cardiotoxicity.

_Diabetes Depletes GATA4 and Increases CHIP in the Mouse Heart—_To determine whether HG-induced GATA4 depletion and cardiomyocyte death translate in vivo, we measured GATA4 and CHIP expression levels in hearts from type 1 or type 2 diabetic mouse models. Type 1 diabetes was induced in 2-month-old FVB mice with STZ. In this model, cardiac GATA4 protein levels started to decrease as early as day 4 after STZ dosing and reached lowest levels at 4 weeks (Fig. 7A–C). STZ 44 ± 7% versus control 100 ± 9.6%, n = 4, p < 0.05). However, semi-quantitative RT-PCR showed that GATA4 mRNA levels remained unchanged in the diabetic heart (lower panel, Fig. 7B). We also measured cardiac GATA4 levels in 4-month-old db/db mice, a type 2 diabetes model. Similarly, GATA4 protein levels but not mRNA levels were reduced in db/db diabetic hearts compared with db/+ nondiabetic controls (Fig. 7, D and E, db/db 67 ± 13% versus db/+ 100 ± 10%, n = 4, p < 0.05). Furthermore, we found that CHIP mRNA levels were markedly increased in STZ and db/db diabetic mouse hearts as determined by RT-PCR (Fig. 8, A and B, STZ 235 ± 9.8% versus control 100 ± 18%; db/db 173 ± 13% versus db/+ 100 ± 9.6%, n = 4, p < 0.01). In contrast, the mRNA levels of MuRF1 and atrogin1 were not changed in STZ diabetic hearts and significantly reduced in db/db diabetic hearts. In summary, diabetes, both type 1 and type 2, diminished GATA4 and increased CHIP, which correlated with our findings in NRVCs, suggesting a novel mechanism that might mediate hyperglycemic cardiotoxicity.

**FIGURE 5.** Overexpression of GATA4 in NRVCs prevented HG-induced GATA4 reduction and myocyte death. NRVCs were infected with Adβgal or AdGATA4 and then exposed to NG (5.5 mM), HG (25 or 40 mM), or mannitol (5.5 mM glucose + 34.5 mM mannitol) for 72 h. GATA4 protein and mRNA levels were determined by Western blot analysis (left panel of A) and RT-PCR (right panel of A), respectively. Myocyte death was determined by propidium iodide (PI) staining (B). PI positive NRVCs were expressed as the percentage of total myocytes counted under phase contrast (C). Shown are representative results of several experiments in triplicate, n = 3, #, p < 0.05, * p < 0.01 versus 5.5 mM glucose.

**DISCUSSION**

More than two-thirds of diabetic patients die from heart disease. A major mechanism proposed for diabetic cardiac damage is oxidative stress that is presumably induced by hyperglycemia (2). The ability of various antioxidants to attenuate diabetic heart failure has been firmly established in animal studies (10, 14, 15). However, clinical trials have not reproduced these results (16, 17). Thus, to reduce diabetic heart failure, further studies are warranted to identify and better understand the molecular targets and signaling mechanisms that mediate diabetic cardiac damage. In this study, we showed that the protein levels of GATA4, a transcription factor essential for cardiomyocyte growth and survival, were markedly diminished in cardiomyocytes by HG and in the hearts of STZ-induced type 1 diabetic mice and db/db type 2 diabetic mice. Our results dem-
onstrate that hyperglycemia-induced GATA4 depletion is likely caused by the increased expression of the E3-ubiquitin ligase CHIP that mediates GATA4 ubiquitination and degradation by the UPS. We also found that overexpression of GATA4 prevented hyperglycemia-induced GATA4 depletion and myocyte death, consistent with previous studies that suggest a cardioprotective role of GATA4 in response to diverse pathological insults, including endotoxin (24), doxorubicin (26, 36), and myocardial infarction (25). In summary, our findings identify a potentially novel mechanism to explain hyperglycemic cardiotoxicity and diabetic cardiomyopathy.

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may not be desirable because the hearts of CHIP knock-out mice show increased sensitivity to ischemia-reperfusion injury (41), which suggests an important role for CHIP in maintaining normal cardiac homeostasis.

Besides hyperglycemia, a number of other pathological insults are also able to deplete GATA4 protein levels in the heart such as endotoxin (24), doxorubicin (36), and myocardial infarction (25). The mechanisms whereby GATA4 is depleted in cardiomyocytes are not necessarily the same under different pathological conditions. For example, doxorubicin-induced GATA4 depletion is mediated by both repressed gene transcription (26) and enhanced protein degradation by the increased UPS activity (42). Thus, proteasomal inhibition not only reverses GATA4 depletion but also attenuates doxorubicin-induced cardiomyocyte death.5 However, because the proteasomal function was somewhat impaired in HG-treated NRVCs as determined by the GFPu reporter or the peptidase activity, further proteasomal inhibition is probably not a viable strategy to reduce HG-induced cardiotoxicity despite the fact that the proteasome inhibitor MG262 can completely reverse GATA4 depletion. Alternative strategies that can enhance GATA4 gene expression or increase the stability of GATA4 protein by post-translational modifications may turn out to be more beneficial in reducing hyperglycemia-induced cardiotoxicity.

In summary, we demonstrate that GATA4 protein levels were markedly reduced in cardiomyocytes by high glucose. The reduction in GATA4 protein levels was associated with an up-regulation of CHIP, an E3 ligase that likely promotes GATA4 ubiquitination and degradation through the UPS. Overexpression of GATA4 prevented GATA4 depletion and attenuated hyperglycemia-induced cardiomyocyte injury. Additionally, GATA4 levels were decreased, and CHIP levels were increased in the hearts of both type 1 and type 2 diabetic mouse models. Together, these results shed new light on the mechanism of hyperglycemic cardiotoxicity and may suggest novel therapeutic strategy for managing diabetic cardiomyopathy.

REFERENCES

1. Bell, D. S. (1995) Diabetes Care 18, 708–714
2. Poomrime, I. G., Parikh, P., and Shannon, R. P. (2006) Circ. Res. 98, 596–605
3. Cooper, M. E. (2004) Am. J. Hypertens. 17, S31–S38
4. Pierce, G. N., and Russell, J. C. (1997) Cardiovasc. Res. 34, 41–47
5. Fiordaliso, F., Li, B., Latini, R., Sonnenblick, E. H., Anversa, P., Leri, A., and Kajstura, J. (2000) Lab. Investig. 80, 513–527
6. Idris, I., Gray, S., and Donnelly, R. (2001) Diabetologia 44, 659–673
7. Cai, L., Li, W., Wang, G., Guo, L., Jiang, Y., and Kang, Y. J. (2002) Diabetes Care 25, 959–968
8. Malhotra, A., Begley, R., Kang, B. P., Rana, I., Liu, J., Yang, G., Moehly-Rosen, D., and Meggs, L. G. (2005) Am. J. Physiol. 289, H1343–H1350
9. Fruittaci, A., Kajstura, J., Chimenti, C., Jakoniuk, I., Leri, A., Maseri, A., Nadal-Ginard, B., and Anversa, P. (2000) Circ. Res. 87, 1123–1132
10. Fiordaliso, F., Bianchi, R., Staszewsky, L., Cuccovillo, I., Doni, M., Laragione, T., Salio, M., Savino, C., Melucci, S., Santangelo, F., Scanziani, E., Masson, S., Ghezzi, P., and Latini, R. (2004) J. Mol. Cell. Cardiol. 37, 959–968
11. Malhotra, A., Kang, B. P., Hashmi, S., and Meggs, L. G. (2005) Mol. Cell. Biochem. 268, 169–173

S. Kobayashi and Q. Liang, unpublished observations.
GATA4 in Hyperglycemic Cardiomyocyte Injury

12. Shizukuda, Y., Reyland, M. E., and Buttrick, P. M. (2002) Am. J. Physiol. 282, H1625–H1634
13. Fiordaliso, F., Leri, A., Ceselli, D., Limana, F., Safai, B., Nadal-Ginard, B., Anversa, P., and Kajstura, J. (2001) Diabetes 50, 2363–2375
14. Ye, G., Metreveli, N. S., Donthi, R. V., Xia, S., Xu, M., Carlson, E. C., and Epstein, P. N. (2004) Diabetes 53, 1336–1343
15. Liang, Q., Carlson, E. C., Donthi, R. V., Kralik, P. M., Shen, X., and Epstein, P. N. (2002) Diabetes 51, 174–181
16. Lonn, E., Yusuf, S., Hoogwerf, B., Pogue, J., Yi, Q., Zinman, B., Bosch, J., Dagenais, G., Mann, J. F., and Gerstein, H. C. (2002) Diabetes Care 25, 1919–1927
17. Sacco, M., Pellegrini, F., Roncaglioni, M. C., Avanzini, F., Tognoni, G., and Nicolucci, A. (2003) Diabetes Care 26, 3264–3272
18. Molkentin, J. D. (2000) J. Biol. Chem. 275, 38949–38952
19. Liang, Q., De Windt, L. J., Witt, S. A., Kimball, T. R., Markham, B. E., and Molkentin, J. D. (2001) J. Biol. Chem. 276, 30245–30253
20. Oka, T., Maillet, M., Watt, A. J., Schwartz, R. J., Aronow, B. J., Duncan, S. A., and Molkentin, J. D. (2006) Circ. Res. 98, 837–845
21. Kakita, T., Hasegawa, K., Iwai-Kanai, E., Adachi, S., Morimoto, T., Wada, H., Kawamura, T., Yanazume, T., and Sasayama, S. (2001) Circ. Res. 88, 1239–1246
22. Maulik, N., Engelman, R. M., Rousou, J. A., Flack, J. E., III, Deaton, D., and Das, D. K. (1999) Circulation 100, 369–375
23. Suzuki, Y. J., Nagase, H., Day, R. M., and Das, D. K. (2004) J Mol. Cell. Cardiol. 37, 1195–1203
24. Chagnon, F., Metz, C. N., Bucala, R., and Lesur, O. (2005) Circ Res. 96, 1095–1102
25. Li, Y., Takemura, G., Okada, H., Miyata, S., Esaki, M., Okada, H., Kamamori, H., Khai, N. C., Maruyama, R., Ogino, A., Minatoguchi, S., Fujiwara, T., and Fujiwara, H. (2006) Circulation 113, 535–543
26. Aries, A., Paradis, P., Lefebvre, C., Schwartz, R. J., and Nemer, M. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 6975–6980
27. Kobayashi, S., Lackey, T., Huang, Y., Bisping, E., Pu, W. T., Boxer, L. M., and Liang, Q. (2006) FASEB J. 20, 800–802
28. Phelps, M., Phillips, A., Darley, M., and Blaydes, J. P. (2005) J. Biol. Chem. 280, 16651–16658
29. Kuzman, J. A., Gerdes, A. M., Kobayashi, S., and Liang, Q. (2005) J. Mol. Cell. Cardiol. 39, 841–844
30. Qian, S. B., McDonough, H., Boelmann, F., Cyr, D. M., and Patterson, C. (2006) Nature 440, 551–555
31. Li, H. H., Kedar, V., Zhang, C., McDonough, H., Arya, R., Wang, D. Z., and Patterson, C. (2004) J. Clin. Invest. 114, 1058–1071
32. Arya, R., Kedar, V., Hwang, J. R., McDonough, H., Li, H. H., Taylor, J., and Patterson, C. (2004) J. Cell Biol. 167, 1147–1159
33. Dong, X., Liu, J., Zheng, H., Glasford, J. W., Huang, W., Chen, Q. H., Harden, N. R., Li, F., Gerdes, A. M., and Wang, X. (2004) Am. J. Physiol. 287, H1417–H1425
34. Rossini, A. A., Like, A. A., Chick, W. L., Appel, M. C., and Cahill, G. F., Jr. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 2485–2489
35. Aasum, E., Hafstad, A. D., Severson, D. L., and Larsen, T. S. (2003) Diabetes 52, 434–441
36. Li, L., Takemura, G., Li, Y., Miyata, S., Esaki, M., Okada, H., Kamamori, H., Khai, N. C., Maruyama, R., Ogino, A., Minatoguchi, S., Fujiwara, T., and Fujiwara, H. (2006) Circulation 113, 535–543
37. Glickman, M. H., and Ciechanover, A. (2002) Physiol. Rev. 82, 373–428
38. Ballinger, C. A., Connell, P., Wu, Y., Hu, Z., Thompson, L. J., Yin, L. Y., and Patterson, C. (1999) Mol. Cell. Biol. 19, 4535–4545
39. Demand, J., Alberti, S., Patterson, C., and Hohfeld, J. (2001) Curr. Biol. 11, 1569–1577
40. Peng, H. M., Morishima, Y., Jenkins, G. J., Dunbar, A. Y., Lau, M., Patterson, C., Pratt, W. B., and Osawa, Y. (2004) J. Biol. Chem. 279, 52970–52977
41. Zhang, C., Xu, Z., He, X. R., Michael, L. H., and Patterson, C. (2005) Am. J. Physiol. 288, H2836–H2842
42. Kumarapeli, A. R., Horak, K. M., Glasford, J. W., Li, J., Chen, Q., Liu, J., Zheng, H., and Wang, X. (2005) FASEB J. 19, 2051–2053