p53 and TIGAR regulate cardiac myocyte energy homeostasis under hypoxic stress

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Despite significant therapeutic advances, heart failure (HF) remains a leading cause of morbidity and mortality (12, 32). Numerous studies (10, 26) have identified altered cardiac energy homeostasis as a consistent feature of HF. Recently, metabolic-based therapies have attracted much interest as a new approach for the treatment of HF. For example, a metabolic modulator, trimetazidine, shifts the energy source from free fatty acid toward predominantly glucose utilization and improves the cardiac function of patients with idiopathic dilated cardiomyopathy (35). Transgenic mice overexpressing the cardiac function of patients with idiopathic dilated cardiomyopathy (35). Transgenic mice overexpressing

by chronic pressure overload or ischemic injury (17, 20). Based on these promising findings, additional insights into the mechanisms regulating cardiac energy metabolism may be helpful for further advancing our treatment regimens.

Cardiac myocytes are known to undergo apoptosis in hypoxia and ischemia-reperfusion (6, 34). Reducing apoptotic cell death and the effect of remodeling after myocardial infarction (MI) have been primary goals, as extensive MI causes severe congestive HF (15). p53 regulates apoptosis, DNA repair, cell cycle progression, and senescence in response to genomic DNA damage, but it also appears to have an expanding role in the heart. Induction of p53 protein by myocardial ischemia is associated with a decreased survival rate, and its induction by treatment with doxorubicin decreases cardiac function (24, 31). Chronic pressure overload induces p53 protein in the heart and decreases angiogenesis and oxygenation through the inhibition of hypoxia-inducible factor (HIF)-1α (30).

We have previously reported that p53 regulates mitochondrial respiration (22). In view of the important role of energy homeostasis in determining the survival of cardiac myocytes (34), we focused on the role of p53 in energy metabolism of myocytes under hypoxic stress simulating myocardial ischemia. We examined glucose metabolism and myocyte apoptosis in response to energetic changes under hypoxia. Our results demonstrate that inhibition of glycolysis is closely involved in the induction of myocyte apoptosis and TP53-induced glycolysis and apoptosis regulator (TIGAR), the gene plays a crucial role in myocyte energy maintenance by regulating glycolysis.

METHODS

Animals. This investigation confirmed with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996). The protocol was approved by the Bioethics Committee of Kyoto Prefectural University School of Medicine.

Materials. The materials used are described in the Supplemental Material.1

Surgical procedures of MI in mice. The operation was performed as previously described (23). The procedures are described in detail in the Supplemental Material.

Cultured neonatal rat cardiac myocytes. Primary cultures of neonatal rat cardiac myocytes were prepared as previously described (21). These methods are described in detail in the Supplemental Material.

Exposure to hypoxia and determination of medium pH. Myocytes were incubated with DMEM containing 0.5% FBS and 2 g/l glucose 12 h before the exposure to hypoxia. Small interfering (si)RNAs for p53 and TIGAR were administered 48 h before hypoxia. To block transcriptional changes induced by p53, we used a chemical p53

1 Supplemental Material for this article is available at the American Journal of Physiology-Heart and Circulatory Physiology website.

1
inhibitor, pifithrin (PFT)-α (13). PFT-α (2 × 10⁻⁵ mol/l) was added to the myocytes 3 h before the exposure to hypoxia. A glycolytic inhibitor, 2-deoxyglucose (2-DG), was added to the myocytes before the exposure to hypoxia. Cardiac myocytes were then transferred to a hypoxic chamber (APM-30D, ASTEC) and maintained at 37°C in a humidified atmosphere to hypoxia. Cardiac myocytes were then transferred to a hypoxic chamber (APM-30D, ASTEC) and maintained at 37°C in a humidified atmosphere to hypoxia.

The pH of the media under hypoxia conditions decreased in a time-dependent manner (0 h: 7.42 ± 0.11, 3 h: 7.38 ± 0.12, 6 h: 7.35 ± 0.14, 9 h: 7.25 ± 0.18, and 12 h: 7.21 ± 0.19). Although the addition of HEPES inhibited the change in pH from 7.42 ± 0.12 to 7.40 ± 0.11, we used media without HEPES in the present study to see the physiological changes in metabolites.

**Histochemical determination of apoptosis.** Histochemical staining of myocytes was performed as previously described (21, 23). This is described in detail in the Supplemental Material.

**Measurement of glucose and lactate levels.** Glucose and lactate levels in the culture media were determined spectrophotometrically at 37°C by measuring quinone absorbance at 585 and 555 nm using the Detaminer GL-E (Kyowa Medics) and Detaminer LA (Kyowa Medics), respectively (34).

**Measurement of D-[1-14C]glucose oxidation.** Glucose oxidation was assessed by measuring the production of 14CO2 from D-[1-14C] glucose (3). This is described in detail in the Supplemental Material.

**Real-time RT-PCR.** Total RNA was isolated from myocytes using TRIzol (Invitrogen) and reverse transcribed with the Super Script III first-strand synthesis system (Invitrogen). Quantitative PCR was performed by a Light Cycler (Roche) using the Fast Start DNA Master PLUS SYBR Green I kit (Roche). Expression levels of target genes were normalized by the expression levels of β-actin. The primer sequences are described in the Supplemental Material.

**Western blot analysis.** For the detection of p53, phosphorylated p53-Ser15, Bax, Bcl-2, HIF-1α, and β-actin, equal amounts of protein were fractionated on Tris-glycine SDS-polyacrylamide gels and subjected to electrophoresis and transferred to a polyvinylidene difluoride membrane (Bio-Rad). Chemiluminescence was detected with ECL Western blot detection kits (Amersham) according to the supplier’s recommendations.

**Real-time RT-PCR.** Total RNA was isolated from myocytes using TRIzol (Invitrogen) and reverse transcribed with the Super Script III first-strand synthesis system (Invitrogen). Quantitative PCR was performed by a Light Cycler (Roche) using the Fast Start DNA Master PLUS SYBR Green I kit (Roche). Expression levels of target genes were normalized by the expression levels of β-actin. The primer sequences are described in the Supplemental Material.

**Transient transfection with plasmid DNA and siRNA.** Rat TIGAR cDNA (NM_001025064) was cloned into the multiple cloning sites of pc topo DNA (Invitrogen) and sequenced for confirmation. Plasmids were transiently transfected into myocytes using Lipofectamine 2000 (Invitrogen) according to the product protocol. The transfection efficiency of pcDNA to myocytes was estimated as 12.2 ± 2.1% (n = 6) on the basis of the transfection efficiency of pEGFP-N1 (Clontech).

**Nonspecific, p53-specific, and TIGAR-specific siRNA duplexes were purchased from Invitrogen. siRNAs were transiently transfected...**
into the myocytes using Lipofectamine RNAiMAX (Invitrogen) according to the product protocol. Myocytes were given fresh media 24 h after transfection, and total RNA was isolated from myocytes 48 h after transfection. The sequences of siRNAs are described in the Supplemental Material.

Assay of fructose-2,6-bisphosphate levels. The fructose-2,6-bisphosphate (Fru-2,6-P2) level was determined by its ability to activate pyrophosphate-dependent phosphofructo-1-kinase (PFK-1) from potato tubers as previously described (1, 36). Briefly, myocytes were homogenized in NaOH (5 × 10⁻² mol/l) and neutralized with acetate to pH 7.0 in the presence of HEPES (2 × 10⁻² mol/l). After being centrifuged at 14,000 g for 5 min, the supernatants were used for assays.

Measurement of high-energy phosphates in cardiac myocytes. Cardiac myocytes were treated with 0.25 ml of 0.6 N ice-cold perchloric acid and centrifuged at 1,000 g for 5 min at 4°C. The supernatant was neutralized with KOH to pH 5.0–7.0 and, after 10 min, was centrifuged at 8,000 g for 5 min at 4°C to remove KClO₄. The supernatant was then used for the assays. ATP and phosphocreatine (PCr) were measured by HPLC (LC-9A, Shimadzu) with a STR ODS-M column (Shimadzu) (34).

Measurement of mitochondrial membrane potential. Loss of mitochondrial membrane potential (ΔΨ) was assessed by the ratio of red to green fluorescence using 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide (JC-1; Molecular Probes). Coverslip-grown myocytes were incubated in PBS containing 5 × 10⁻⁶ mol/l JC-1 at 37°C for 5 min and then observed by confocal microscopy (FV1000, Olympus). Fluorescence was viewed at 530 and 590 nm using excitation at 488 nm (34). Tetramethylrhodamine ethyl ester (TMRE; excitation: 550 nm and emission: 580 nm; 500 nM) fluorescence was also monitored to examine the changes of ΔΨ (25).

Statistical analysis. Data are expressed as means ± SE of at least six samples derived from more than six separate experiments. Differences were analyzed by one-way ANOVA combined with the Fisher post hoc test. P values of <0.05 were considered to indicate statistical significance.

RESULTS

Hypoxia-induced p53 regulates glycolysis. We first examined the effect of hypoxic stress on cardiac p53 expression. Expression of p53 was markedly increased 24 h after MI in

![Fig. 2. TP53-induced glycolysis and apoptosis regulator (TIGAR) is induced by hypoxia and regulated by p53. A: Western blot analysis for cardiac TIGAR protein after MI in WT and p53 knockout (p53KO) mice. TIGAR protein was induced after coronary ligation in WT mice but not in p53KO mice. B–D: time-dependent expression of TIGAR mRNA (top) and protein (bottom) in hypoxic cardiac myocytes as well as effects of hypoxia, the p53 inhibitor pifithrin (PFT-α), and p53 siRNA treatment on TIGAR mRNA (3 h after hypoxia) and expression of p53 and TIGAR proteins (6 h after hypoxia). Hypoxia induced TIGAR mRNA and protein (B). Treatment with p53 siRNA or PFT-α blocked the expression of TIGAR mRNA and protein (C and D). **P < 0.01 vs. 0 h in WT mice (A); *P < 0.05 vs. normoxia with NS siRNA (C) or control (B and D); #P < 0.05 vs. hypoxia with NS siRNA (C) or control (D).](http://ajpheart.physiology.org/)

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wild-type (WT) mice (Fig. 1A). Apoptotic myocytes were increased in the border region of ischemia after 24 h in WT mice compared with p53-deficient [p53 knockout (p53KO)] mice (12.9% vs. 7.5%, *P < 0.01; Supplemental Fig. 1). We next examined the role of p53 in apoptosis and glucose metabolism using cardiac myocytes as previously reported (5, 7, 30, 33, 34). Expression of p53 and phosphorylated p53 was markedly induced during hypoxia; however, expression of Bcl-2 and Bax protein was not affected up to 9 h of hypoxia (Fig. 1B). Treatment with p53 siRNA abolished hypoxia-mediated expression of p53, whereas NS siRNA did not affect it (Fig. 1C). Staining for apoptotic nuclei and cellular actinin showed that hypoxia induced apoptosis in a time-dependent manner (baseline: 2.5% to 19% at 12 h; Fig. 1D and Supplemental Fig. 2), whereas inhibition with p53 siRNA significantly reduced the percentage of myocyte apoptosis (Fig. 1D).

We next measured glucose utilization and lactate production in the incubation medium as markers of metabolic activity during hypoxia. Glucose utilization in control NS siRNA-treated myocytes was markedly upregulated by hypoxia and further increased by treatment with p53 siRNA (55% increase vs. NS siRNA at 6 h, *P < 0.01; Fig. 1E). In parallel, lactate production was also elevated by hypoxia and further increased by treatment with p53 siRNA (73% increase vs. NS siRNA at 6 h, *P < 0.01; Fig. 1F).

Glycolysis regulator TIGAR is induced by p53. p53 has been shown to coordinately increase oxidative phosphorylation and decrease glycolysis (1, 22). The TIGAR gene has a p53-responsive element and shares functional similarity with fructose-2,6-bisphosphatase (FBPase-2), which degrades Fru-2,6-P2, a potent activator of PFK-1 that governs the entry of glucose into the glycolytic pathway. Thus, the induction of TIGAR by p53 serves to inhibit glycolysis.

We found that the expression of TIGAR protein was markedly induced 24 h after MI in WT mice but not in p53KO mice (3.1-fold at 24 h from 0 h in WT mice, *n = 6, *P < 0.01; Fig. 2A). Hypoxic stress to cardiac myocytes caused a significant accumulation in p53 and TIGAR mRNA levels in a time-dependent manner (1.4-fold at 6 h from baseline, *P < 0.05; Fig. 2B, top) followed by increases in protein levels (Fig. 2B, bottom). Further association between p53 and TIGAR was confirmed in cardiac myocytes under normoxic conditions using Nutlin-3, a pure p53 inducer (Supplemental Fig. 3A). Using D-[1-14C]glucose, we were able to show a decrease in glucose metabolism (44% vs. control; Supplemental Fig. 3B) after the induction of p53 and TIGAR.

Because siRNA-mediated inhibition of p53 increased the rates of glucose utilization and lactate production with a maximal effect 6 h after exposure (Fig. 1, E and F), we examined its effect on TIGAR expression using myocytes.
under the same conditions. Hypoxia-mediated expression of TIGAR mRNA was inhibited by both p53 siRNA (Fig. 2C) and the p53 inhibitor PFT-α (Fig. 2D), suggesting that its expression was dependent on p53. The increase in TIGAR protein levels by hypoxia was also blocked by p53 siRNA (48% decrease; Fig. 2C) or PFT-α (21% decrease; Fig. 2D). These data indicate that TIGAR was transcriptionally regulated by p53 under hypoxic condition.

Fru-2,6-P₂ is augmented by inhibition of TIGAR. As TIGAR decreased the level of glycolysis by activating Fru-2,6-P₂ (1), we examined its levels in hypoxic cardiac myocytes. We found that Fru-2,6-P₂ levels were increased after 6 h of hypoxia (15 ± 1.6% vs. normoxia, P < 0.05; Fig. 3A) and that treatment with p53 siRNA or PFT-α further increased Fru-2,6-P₂ levels (15 ± 1.9% and 13 ± 1.4%, respectively, P < 0.05; Fig. 3, A and B). We further examined the effect of TIGAR siRNA on glycolysis. Treatment with TIGAR siRNA reduced its mRNA and protein levels 81% and 85% respectively (n = 6 each vs. control NS siRNA; Fig. 3C). TIGAR siRNA significantly increased Fru-2,6-P₂ (18 ± 1.8%, P < 0.05; Fig. 3D) and markedly upregulated glucose utilization and lactate production during hypoxia (76% and 86% increases at 6 h, respectively, P < 0.01; Fig. 3, E and F), whereas myocyte apoptosis as evaluated by nuclei fragmentation was significantly reduced by TIGAR siRNA (24% inhibition at 6 h of hypoxia, P < 0.05; Fig. 3G). We also examined the effect of the glycolytic inhibitor 2-DG. The addition of 2-DG to the incubation medium abolished the protective effect of TIGAR siRNA on apoptosis (Fig. 3H).

Overexpression of TIGAR reduced glycolysis and increased myocyte apoptosis. We investigated the effect of TIGAR overexpression on glycolysis and myocyte apoptosis. Transfection of pcDNA TIGAR (300 and 1,000 ng/1 × 10⁶ cells) to cardiac myocytes induced TIGAR protein by 1.7 ± 0.2- and 2.1 ± 0.3-fold (n = 6 each, respectively; Fig. 4A) and decreased Fru-2,6-P₂ content (39% decrease at 1,000 ng/1 × 10⁶ cells; Fig. 4B). We used 1,000 ng of transfection to 1 × 10⁶ cells in the following experiments.

TIGAR overexpression resulted in the enhancement of hypoxia-mediated myocyte apoptosis (23.2 ± 1.8% after 12 h of hypoxia; Fig. 4C) and inhibition of glucose utilization and lactate production (32.2 ± 2.8% and 38.1 ± 2.9% after 12 h of hypoxia; Fig. 4, D and E). Next, we measured high-energy phosphates of myocytes as an additional measure of the bioenergetic homeostasis of cells. PCr content was significantly reduced after 6 h of hypoxia by TIGAR overexpression (Fig. 4F) without changes in ATP levels (Fig. 4G). Taken together, these findings suggested that TIGAR enhances hypoxia-in-
duced myocyte apoptosis and that inhibition of glycolysis is closely involved in the mechanism of myocyte apoptosis.

High-energy phosphates are preserved by inhibition of TIGAR. We measured ATP and PCr in myocytes after an exposure to hypoxia for 6 h. Although hypoxia did not affect the ATP content (Fig. 5, A–C) but markedly reduced the PCr content (~65% decrease; Fig. 5, D–F). Treatment with p53 siRNA or PFT-α prevented this hypoxia-mediated decrease in PCr contents to 79% and 74% of normoxia controls, respectively (Fig. 5, D and E). Treatment with siRNA against TIGAR also prevented the reduction in PCr to 75% of the normoxia control (Fig. 5F), again suggesting that the activity of p53 is mediated through TIGAR.

\( \Delta \Psi \text{ is preserved by inhibition of p53 and TIGAR.} \) As mitochondrial membrane depolarization is essential for apoptotic cell death, we examined the stability of \( \Delta \Psi \) using a sensitive probe, JC-1 (5, 7, 34). Control myocytes showed red to orange mitochondrial staining, indicative of normal high \( \Delta \Psi \) (Fig. 6, A, E, and I). In contrast, 6 h of hypoxia caused green fluorescence, indicating the loss of \( \Delta \Psi \) (Fig. 6, B, F, and J). Quantitative analyses of the ratio of red to green fluorescence showed that 6 h of hypoxia significantly decreased \( \Delta \Psi \) (26%, 23%, and 27% of normoxia controls, respectively, \( P < 0.01 \); Fig. 6, D, H, and L) and that the inhibition of p53 by siRNA or PFT-α partially prevented the hypoxia-induced loss of \( \Delta \Psi \) and maintained it at 60% and 46% of normoxia controls, respectively (Fig. 6, C, D, G, and H). Knockdown of TIGAR by siRNA similarly prevented the loss of \( \Delta \Psi \) (Fig. 6, K and L).

Inhibition of p53 and TIGAR contributes to the maintenance of \( \Delta \Psi \) by augmenting glycolysis. TMRE fluorescence is dependent on \( \Delta \Psi \). Intensities of TMRE showed a time-dependent decrease of \( \Delta \Psi \) during hypoxia, whereas inhibition of p53 or TIGAR significantly attenuated the hypoxia-induced loss of \( \Delta \Psi \) (Fig. 7A). To determine whether glycolysis is involved in regulating \( \Delta \Psi \), we examined the effect of 2-DG. Treatment with 2-DG completely blocked the p53 and TIGAR siRNA-mediated preservation of \( \Delta \Psi \) (Fig. 7B), linking glycolysis through the preservation of PCr to the stabilization of \( \Delta \Psi \). Thus, these data showed that the increase in glycolysis, which can be inhibited by p53 through TIGAR, is important for preserving \( \Delta \Psi \) and preventing cell death.

**DISCUSSION**

Our present study focused on the relationships among the p53/TIGAR system, energy homeostasis, and apoptosis in cardiac myocytes under hypoxic stress. We found that 1) the expression of TIGAR is induced by p53 in hypoxic myocytes,

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**Fig. 5**. The ratio of PCr to ATP is improved by inhibition of p53 and TIGAR. ATP and PCr contents were measured in cardiac myocytes exposed to hypoxia for 6 h. A–F: hypoxia did not affect ATP contents (A–C), whereas PCr contents were decreased by hypoxia, and p53 siRNA (D), PFT-α (E), or TIGAR siRNA (F) attenuated the decline of PCr. \( **P < 0.01 \) vs. normoxia with NS siRNA (D and F) or normoxia control (E); \( ###P < 0.01 \) vs. hypoxia with NS siRNA (D and F) or hypoxia control (E).
2) TIGAR inhibits hypoxia-induced glycolysis and lowers PCr levels with associated loss of ΔΨ, and 3) TIGAR-mediated inhibition of glycolysis is closely involved in the induction of myocyte apoptosis.

Role of p53 in myocyte apoptosis. In the ischemic myocardium, p53-dependent myocyte apoptosis has been observed (18), whereas the contribution of p53 to bioenergetic changes has not been characterized. Using various models, there have been contrasting reports (2, 37) that have shown a p53-independent mechanism in ischemic myocyte apoptosis. Therefore, there is a need for further understanding the common biology underlying these disparate findings, and a recent report (24) has demonstrated that p53 is involved in ischemia-induced myocyte apoptosis, which causes cardiac rupture, indicating potentially important clinical implications of unmoderated p53 activity.

Modulation of ΔΨ. For the execution of apoptosis, mitochondria undergo two major alterations. One is the permeabilization of the outer mitochondrial membrane, and the other is the loss of the electrical gradient (membrane depolarization). The permeabilization of the outer membrane is tightly regulated by a member of the Bcl-2 family and involves the conformational changes in proapoptotic family members such as Bax. Membrane depolarization is mediated by the mitochondrial permeability transition pore (mPTP). Prolonged mPTP opening leads to a compromise of the outer mitochondrial membrane (28). Inhibition of the opening of the mPTP and preventing the dissipation of ΔΨ represents a promising strategy to prevent HF (5, 9). We found that Bcl-2 and Bax protein levels did not change significantly up to 9 h of hypoxia (Fig. 1B), despite the significant change in ΔΨ before the alteration of Bcl-2 and Bax protein (Fig. 6). Because maintaining ΔΨ through the import of glycolytic ATP reduced apoptotic cell death under anoxic conditions (25), we evaluated the rapid response of energy status and ΔΨ under hypoxic conditions before significant activation of Bax protein. As shown in Fig. 7, glucose in the culture media was essential for maintaining ΔΨ. Maintaining ATP levels by glycolysis may contribute to myocyte protection against hypoxic injury.
Glycolytic ATP, acidosis, and mPTP inhibition. The high energy of PCr is converted to ATP in the myofibrils, which is an important short-term reserve energy source to maintain cellular energy homeostasis. Although it has been shown that increased glycolytic ATP is protective against ischemia (29) and subsequent reperfusion injury (8, 11), concerns have been raised that excessive acceleration of glycolysis may exacerbate myocardial injury because of metabolic byproducts (lactate and acidosis) and acidosis (H⁺). As acidosis is known to prevent mPTP opening by blocking Ca²⁺ binding to adenine nucleotide translocase and displacing cyclophilin from it (27), moderate enhancement of acidosis would protect myocytes during ischemia. Furthermore, Cohen et al. (4) reported that maintaining acidosis during the first minutes of reperfusion is critical to inhibit mPTP opening. Considering that the incubation media of myocytes exposed to hypoxia (6 h) became slightly acidic (pH 7.35), H⁺ production from glycolysis might be involved in our hypoxic model. We assumed that modulation of p53 or TIGAR could enhance glycolysis physiologically, which protected myocytes without a detrimental amount of metabolites. Further studies would be required to define the precise interactions between glycolysis and acidosis during myocyte ischemia.

TIGAR in myocytes. From an analysis of structural and biochemical studies, TIGAR has been suggested to function as FBPase-2 (16). In the pathway of glycolysis, 6-phosphofructo-2-kinase (PFK-2)/FBPase-2 is a bifunctional enzyme with both kinase and bisphosphatase activities. PFK-2/FBPase-2 regulates intracellular Fru-2,6-P₂, a potent positive allosteric effector of PFK-1, which is a rate-limiting enzyme of glycolysis. TIGAR increases the degradation of Fru-2,6-P₂, thereby decreasing the activity of PFK-1, which then inhibits glycolysis. As shown in Supplemental Fig. 3, even under aerobic conditions, induction of p53 enhanced the TIGAR protein. Glucose utilization, as examined by d-[1-¹⁴C]glucose oxidation, was significantly decreased after the induction of p53. In our investigation with cultured hypoxic myocytes, inhibition of TIGAR induced glycolysis (Fig. 3, D–F), whereas overexpression of TIGAR reduced glycolysis (Fig. 4, B–E). Modulation of TIGAR directly affected myocyte energetics and viability (Figs. 3G, 4, C, F, and 4G, and 5, A–F).

Although the transfection efficiency of pcDNA TIGAR to myocytes was low and was estimated as 12.2 ± 2.1%, we could observe a 2.1-fold increase in TIGAR protein levels, resulting in a significant reduction in Fru-2,6-P₂ levels and glycolysis (Fig. 4). These findings suggest that the induction of TIGAR plays a critical role in metabolic homeostasis under hypoxic conditions. Further studies using virus-mediated TIGAR overexpression in cardiac myocytes might reveal more prominent effects on cardiac energy homeostasis.

Metabolic modulation and apoptosis in the failing heart. Metabolic modulation during myocardial ischemia is a new option for patients with acute ischemia or HF, and specific drugs that induce a shift from free fatty acids toward predominantly glucose utilization have been designed. Although three agents for metabolic alteration (trimetazidine, ranolazine, and perhexiline) have well-documented anti-ischemic effects (14), these drugs still require an establishment of safety issues for wide clinical use (19). Recently, it has been reported that a chronic shift of myocardial substrates induced molecular remodeling of multiple metabolic pathways and reduced metabolic flexibility, which rendered the heart susceptible to contractile dysfunction (38).

Flexible balancing between aerobic respiration and glycolysis against myocardial stress is a key for metabolic modulation. p53, a well-known stress response tumor suppressor, may be a new candidate for the treatment of ischemic myocardium or HF. Especially, modulation of the p53/TIGAR system might be a specific treatment for metabolic switching.

Conclusions. In summary, our data demonstrate that p53 induces TIGAR to inhibit glycolysis and energy metabolism in hypoxic myocytes and that inhibition of the p53/TIGAR system significantly reduces hypoxia-induced apoptotic cell death. Modulation of p53 and TIGAR may present a novel strategy for myocyte energy maintenance and a potential therapeutic window for ischemic heart disease.
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No conflicts of interest, financial or otherwise, are declared by the author(s).

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