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ATF3 negatively regulates adiponectin receptor 1 expression

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ABSTRACT

Adiponectin is an adipocyte-derived hormone that has antidiabetic and antiatherogenic effects through two membrane receptors, adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2). Although it has been reported that the expression of AdipoR1 and AdipoR2 is regulated under physiological and pathophysiological states, their regulation is largely unknown. Previously, we demonstrated that endoplasmic reticulum (ER) stress or obesity-inducible ATF3 negatively regulates the expression of adiponectin and AdipoR2. Here, we investigated the regulation of another adiponectin receptor, AdipoR1 by ATF3, to determine if ATF3 may contribute to impairment of adiponectin signaling by repressing the expression of both adiponectin and adiponectin receptors. We found that treatment with thapsigargin, a stimulator of ATF3 expression as an inducer of ER stress, decreased AdipoR1 expression in insulin-sensitive cells (HepG2, C2C12) and insulin secreting cells (MIN6N8). Furthermore, overexpression of lentivirus carrying-ATF3 decreased AdipoR1 expression in those cells, demonstrating that ATF3 downregulates AdipoR1 expression. Next, we investigated the effects of ATF3 on human AdipoR1 promoter activity and identified an ATF3-responsive region in the promoter. Both thapsigargin treatment and ATF3 expression repressed AdipoR1 promoter activity. Transfection studies using mutant constructs containing 5′-deletions in the human AdipoR1 promoter revealed that putative ATF/CRE site is located between the −248 and −224, TGACGCGG. Chromatin immunoprecipitation assay demonstrated that ATF3 directly binds to human AdipoR1 promoter spanning from −248 to −224. Finally, deletion of the putative ATF/CRE site abrogated ATF3-mediated transrepression of the AdipoR1 promoter. Importantly, ATF3 expression was increased in hyperglycemia or TNF-α-treated C2C12 cells in which AdipoR1 expression was decreased, suggesting that ATF3 may contribute to downregulation of AdipoR1 by hyperglycemia and TNF-α. Collectively, these results demonstrate that ATF3 negatively regulates human AdipoR1 expression via binding to an ATF3-responsive region in the promoter, which plays an important role in attenuation of adiponectin signaling and induction of insulin resistance.

1. Introduction

Adiponectin, an adipokine secreted by the white adipose tissue, plays an important role in regulating glucose and lipid metabolism and controlling energy homeostasis in insulin-sensitive tissues. Adiponectin exerts its effects through adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2) [1,2]. AdipoR1 is expressed ubiquitously, most abundantly in skeletal muscle, whereas AdipoR2 is predominantly expressed in the liver. AdipoR1 is a high-affinity receptor for globular C-terminal domain of adiponectin with very low affinity for full-length protein of adiponectin, whereas AdipoR2 has intermediate affinity for both forms of adiponectin. Downregulation of both adiponectin and its receptors is associated with insulin resistance and increased risks of type 2 diabetes. The regulation of AdipoR1 and AdipoR2 was reportedly regulated under various physiological and pathophysiological states. Insulin repressed the mRNA expression of AdipoR1 and AdipoR2 via activation of PI3-kinase and inactivation of Foxo1 [3]. Growth hormone was found to upregulate AdipoR2 in adipocytes in vitro [4]. LXR agonists increased the expression of AdipoR1and AdipoR2 in human macrophages, whereas agonists of PPARα and PPARγ only increased the expression of AdipoR2 [5]. Furthermore, the expression of adiponectin receptors was reportedly decreased in obesity or diabetic states, where adiponectin signaling was impaired. The expression levels of both AdipoR1 and AdipoR2 were significantly decreased in adipose tissue of ZDF (Zucker diabetic rats), probably in part because of obesity-linked hyperinsulinemia [6]. The expression of both AdipoR1 and AdipoR2 was also significantly lower in the liver of obese (LepOb and LepDb) mice when compared to wild-type lean animals, while AdipoR1 gene expression was significantly decreased in the

Abbreviations: AdipoR1, adiponectin receptor 1; AdipoR2, adiponectin receptor 2; ATF3, activating transcription factor 3; ER, endoplasmic reticulum; TNF-α, tumor necrosis factor-α; CREB, cAMP responsive element-binding protein; CRE, CREB responsive element; ChIP, chromatin immunoprecipitation.

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pancreas of both LepOb and LepDb mice [7]. In the case of humans, the expression and regulation of adiponectin receptors are associated with type 2 diabetes. In a study of a Mexican-American population, the expression levels of AdipoR1 and AdipoR2 in skeletal muscles and the plasma adiponectin concentration were lower in people with a family history of type 2 diabetes than in those without such a family history [8]. Therefore, obesity decreases both adiponectin levels and AdipoR1/R2 expression, thereby reducing adiponectin sensitivity and leading to insulin resistance.

Activating transcription factor 3 (ATF3) is a member of the ATF/cAMP responsive element-binding family of proteins that acts as a stress-inducible transcriptional repressor [9]. ATF3 has been shown to be induced in cells exposed to a variety of physiological and pathological stimuli, including carbon tetrachloride exposure, anti-cancer drugs, proteasome inhibitors, genotoxic agents, homocysteine and ischemia–reperfusion. ATF3 is also induced in response to endoplasmic reticulum stress or amino acid deprivation [10]. Activated ATF3 can either homodimerize and repress transcription of various promoters with ATF sites or heterodimerize with bZip proteins, c-jun, Jun B, ATF2, or gadd153/CHOP10 (C/EBP homologous protein) and activate transcription of target genes [9]. We recently demonstrated that ATF3 negatively regulates both adiponectin gene expression in adipocyte cells [11] and AdipoR2 in liver cells [12], suggesting that ATF3 plays a critical role in the development of insulin resistance in obesity and type 2 diabetes. Because ATF3 is an ER stress-inducible factor, we also argued that obesity related ER stress may influence the development of insulin resistance, at least in part, by transcriptional repression of the activity of ATF3.

In this study, to further verify that ATF3 contributes to attenuation of adiponectin signaling by repressing the expression of both adiponectin receptors and adiponectin under obesity or diabetic states, we investigated the regulation of another adiponectin receptor, AdipoR1, by ATF3. To accomplish this, we examined the effect of ATF3 overexpression on AdipoR1 in insulin-sensitive cells (HepG2, C2C12) and insulin secreting cells (MIN6N8), and analyzed human AdipoR1 promoter to identify ATF3-responsive site.

2. Materials and methods

2.1. Cell culture

Human hepatocyte HepG2 cells, C2C12 myocytes, and pancreatic MIN6N8 cells were cultured in Dulbecco's modified Eagle's medium containing glucose (Invitrogen, Carlsbad, CA) supplemented with 10% (v/v) fetal bovine serum (Gibco BRL, Gaithersburg, MD), 100 IU/ml penicillin, and 100 μg/ml streptomycin (Gibco Life Technologies, Grand Island, NY). To investigate the effect of ATF3 on AdipoR1 expression, the cells were treated with 1.0 μM thapsigargin (Sigma, St. Louis, MO) for 24 h.

2.2. Overexpression of lentiviral ATF3

Lentiviruses expressing ATF3 were constructed by the Macrogen LentiVector Institute (Seoul, Korea). Briefly, the pcDNA3-ATF3 vector was digested with XbaI/EcoRI, and inserted into a lentiviral vector (Lenti-mCMV-GFP-IRES-puro). The recombinant vector was subsequently transfected into 293T cells using Lipofectamine Plus (Invitrogen), and culture supernatant containing viral particles was harvested after 48 h transfection, clarified with a 0.45 μm membrane filter (Nalgene, NY), designated Lenti M1.4-ATF3/GFP. Titers were determined by p24 ELISA or infection into HeLa cells.

To infect HepG2 cells, HepG2 cells were plated at a density of 1×10^6 cells/well in a 12-well plate and infected with different lentiviral vectors (Lenti M1.4-GFP, Lenti M1.4-ATF3/GFP) in the presence of 6 μg/ml of polybrene. At 16–18 h post infection, the growth media was replaced. The GFP expression of transduced cells was observed under fluorescence microscope (LSM510, Carl Zeiss, Germany).

2.3. Isolation of human AdipoR1 promoter

The regions of the human AdipoR1 promoter corresponding to nucleotides −2069 to −125 and −1076 to −125 were amplified by PCR from human genomic DNA and inserted into the NheI/XhoI sites in pGL3 basic (Promega, Madison, WI) using the sense primers 5′-GGGCTAGCATGGTTTTGAGTTAACAGCC-3′ [for P(-2069)/Luc], 5′-GGGCTAGCTTGCTTTTCTGCTTTTCAGC-3′ [for P(-1076)/Luc], and antisense primer 5′-GGCTCGAGCGGTCCCCCGCGCTACATCC-3′. Deletions in the 5′-flanking regions of the promoter were constructed by PCR using pairwise combinations of the sense primers 5′-GGGCTAGCCCTTGGGGACAGAGGAGATG-3′ [for P(-574)/Luc], 5′-GGGCTAGCCGAGAGGGCCTAGGATTGGA-3′ [for P(−294)/Luc], 5′-GGGCTAGCCGGGGAGGGCGCTGAAGATC-3′ [for P(−200)/Luc] and the antisense primer 5′-GGCTCGAGCGGTCCCCCGCGCTACATCC-3′. Deletion mutant reporter lacking a putative ATF3 binding site was constructed in the P(−294)/Luc reporter by the two step PCR method.

2.4. Transient transfection and luciferase assay

HepG2 cells grown in six-well plates were transiently transfected with human AdipoR1 promoters and ATF3 expression plasmid using Lipofectamine reagent (Invitrogen) according to the manufacturer's instructions. After transfection for 24 h, the cells were lysed in reporter lysis buffer (Promega) and the luciferase activity was measured using a Luciferase Assay System (Promega). To normalize the transfection efficiency, a pCMV-ß gal was included in each transfection as an internal control plasmid and the luciferase activity was normalized against ß-galactosidase activity in each transfection.

2.5. Western blot

The cells were lysed in PRO-PREP lysis reagent according to the manufacturer's protocol (Intron Biotechnology, Sungnam, Korea). Lysed samples were centrifuged at 12,000*g* for 10 min, after which equal amounts of protein were separated by 10% SDS/PAGE, transferred to polyvinylidene difluoride membranes, and incubated with primary antibodies in blocking solution (5% skim milk in phosphate buffer, pH 7.2).

2.6. Reverse transcription (RT)-PCR

Total RNA was extracted using QIAzol lysis reagent (Qiagen, Gaithersburg, MD). The mRNA in the samples was reverse transcribed using a SuperscriptII™ First Strand Kit (Invitrogen). The resulting cDNA was amplified by PCR using the primer pairs human AdipoR1 F (5′-CATCTGGACCCATCTGCTTG-3′) and R (5′-AGGAGAAGCTGAGGCAGAGC-3′) and ATF3 F (5′-AGTGAGTGCTTCTGCCATCG-3′) and ATF3 R (5′-GCAGAGGTGCTTGTTCTGGA-3′). The housekeeping ß-actin gene was amplified using the sense primer 5′-ATGAGGAGGAGATCACTGC-3′ and the antisense primer 5′-CTGCGCAAGTTAGGTTTTGT-3′.

2.7. Chromatin immunoprecipitation (ChIP)

Chromatin immunoprecipitation was conducted using a ChIP Assay Kit (Upstate Biotechnology, Lake Placid, NY) according to the manufacturer's protocol, modified as previously described [11]. HepG2 cells were incubated in the presence of thapsigargin for 24 h or left unstimulated. Immunoprecipitated DNA was ampli-

fied by PCR using primers specific for the AdipoR1 promoter, sense primer 5′-CGAGAGGGCCTAGGATTGGA-3′, antisense primer 5′-GGCCTGCGGCCGAGCGGCCC-3′.

2.8. Statistical analysis

All data are expressed as means ± SEM. ANOVA was used to identify significant differences between groups and was performed using the Duncan test. A $P < 0.05$ was considered statistically significant. All experiments were conducted at least three times.

3. Results

3.1. ATF3 decreases AdipoR1 expression in insulin-sensitive cells and insulin secreting cells

To evaluate the regulation of AdipoR1 by ATF3, we first examined the effect of ATF3-inducer, thapsigargin, on AdipoR1 expression in the insulin-sensitive cells, HepG2 and C2C12, and insulin secreting cells, MIN6N8. As shown in Fig. 1A, treatment with thapsigargin decreased AdipoR1 expression concomitant with an increase in ATF3 expression in those cells. To confirm the downregulation of AdipoR1 by ATF3, we investigated the effect of ATF3 overexpression on AdipoR1 expression in HepG2, C2C12 and MIN6N8 cells. We introduced a lentivirus vector carrying-ATF3 into the cells and then analyzed AdipoR1 expression by RT-PCR. As expected, infection of lenti-ATF3 increased ATF3 expression, whereas it resulted in decreased AdipoR1 expression (Fig. 1B). Taken together, these results demonstrated that ATF3 negatively regulated AdipoR1 expression in insulin-sensitive cells and insulin secreting cells.

3.2. ATF3 decreases the activity of the human AdipoR1 promoter

Next, we examined the effect of ATF3 on the promoter activity of the human AdipoR1. To accomplish this, the −2069 to −125 bp and −1076 to −125 bp (relative to translation initiation site) of the 5′-flanking regions of the human AdipoR1 gene were isolated by PCR and then inserted into a pGL3 basic vector, designated P(−2069)/Luc and P(−1076)/Luc. These promoter-reporters were then transiently transfected into HepG2 cells with ATF3 expression vector, after which the luciferase activities were examined. As shown in Fig. 1C, expression of ATF3 decreased the promoter activities of both the P(−2069)/Luc and P(−1076)/Luc reporters. We also examined the effects of thapsigargin on the promoter activity of human AdipoR1. Thapsigargin treatment decreased the promoter activities of both reporters (Fig. 1D). Taken together, these data indicate that ATF3 negatively regulates the expression of human AdipoR1 by repressing its promoter activity.

3.3. Identification of the ATF3-responsive region

We next identified the ATF3-responsive region in the AdipoR1 promoter. To accomplish this, we produced 5′-serial deleted constructs from P(−1076)/Luc, and the deleted constructs were transfected into HepG2 cells. As shown in Fig. 2A, the deletion of nucleotides −1076 to −294 did not affect the transrepression of the AdipoR1 promoter by ATF3. However, increasing the deletion to −200 resulted in a nearly complete loss of reduction by ATF3 (Fig. 2A), suggesting that the ATF3-responsive region is located between nucleotides −294 and −200.

A computer-assisted search of the 95-bp region using TFSEARCH revealed two putative ATF/CREB responsive elements (ATF/CRE), TGACGCGG (−248/−241 and −231/−224) (Fig. 2B). The site is similar to that of human AdipoR2 promoter (Fig. 2C). To determine if ATF3 is capable of binding to these putative ATF/CRE in vivo, we performed chromatin immunoprecipitation (ChIP) with thapsigargin-treated or untreated HepG2 cells. The chromatins that were immunoprecipitated with ATF3 antibody were amplified by PCR using AdipoR1 promoter-specific primers covering the putative ATF/CRE site from −248 to −224 bp. As shown in Fig. 2D, a 135 bp PCR product was generated with the ATF3 antibody in HepG2, whereas no PCR product was observed

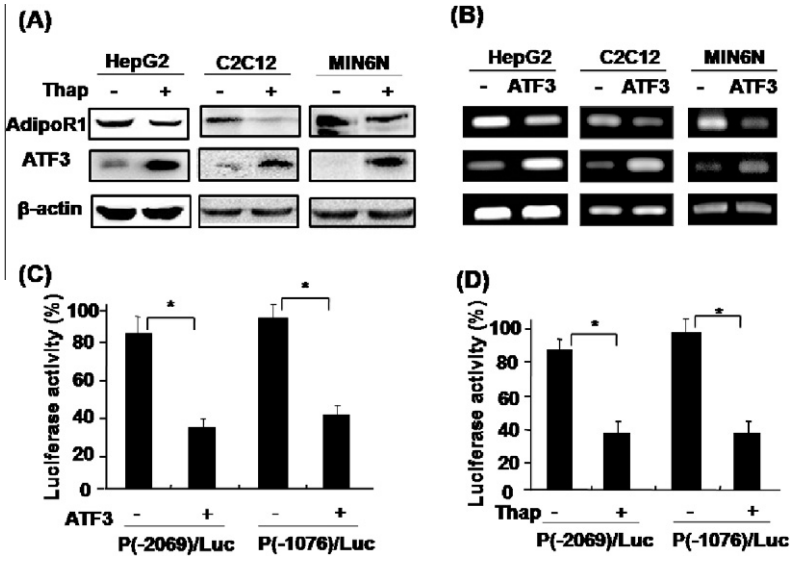

Fig. 1. ATF3 downregulates AdopoR1 expression in insulin-sensitive cells and insulin secreting cells. (A) Thapsigargin treatment decreased the AdipoR1 level. The cells were treated with 1.0 μM thapsigargin for 24 h and the cell lysates were subjected to Western blot. (B) Lentiviral-ATF3 overexpression reduced AdipoR1 mRNA. The cells were infected with lentivirus carrying GFP and ATF3 at a multiplicity of infection 5 and then incubated for 48 h. Total RNA was prepared from lentivirus-infected cells and subjected to RT-PCR using AdipoR1-specific primer to determine AdipoR1 mRNA. (C) ATF3 reduced human AdipoR1 promoter activity. The human AdipoR1 promoters containing −2069/−125 and −1076/−125 were isolated by PCR and inserted into the pGL3 basic reporter plasmid. The reporter constructs, which were designated P(−2069)/Luc and P(−1076)/Luc, were transfected into HepG2 with ATF3 expression vector. After transfection for 24 h, the luciferase activities were measured. Luciferase activities are presented relative to the observable activity from the P(−1076)/Luc. (D) Thapsigargin repressed AdipoR1 promoter activity. Both P(−2069)/Luc and P(−1076)/Luc reporters were transfected into HepG2 and then treated with thapsigargin for 24 h. Luciferase activities are presented relative to the observable activity from P(−1076)/Luc. The asterisks indicate a P value <0.05 for the bracketed comparisons.

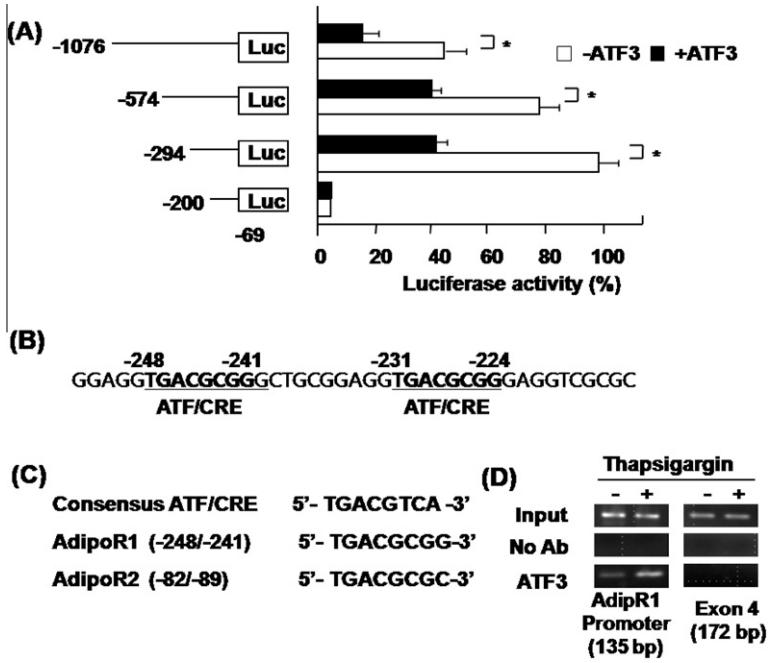

Fig. 2. The ATF/CRE is located between nucleotides −248 and −224. (A) 5′-serial deletions of the human AdipoR1 promoters were constructed using PCR, and the deleted promoters were transfected into HepG2 cells with ATF3 expression vector. The asterisks indicate a *P* value <0.05 for the bracketed comparisons. (B) Schematic representation of human AdipoR1 promoter spanning from −248 to −224. The putative ATF/CREs are shown in bold. (C) Comparison of ATF/CRE of human AdipoR1 promoter with consensus ATF/CRE or human AdipoR2 promoter. (D) ATF3 binds to the AdipoR1 promoter in vivo. HepG2 cells were incubated with thapsigargin for 24 h, and ChIP was performed with or without ATF3 antibody.

when primers for sequences in exon 4 were used. Furthermore, the PCR product was increased to a greater degree following thapsigargin treatment, demonstrating that ATF3 interacts directly with the human AdipoR1 promoter.

To verify that the putative ATF/CRE is crucial for the ATF3-mediated transrepression of the AdipoR1 promoter, we deleted the site within the context of the −294 bp promoter (Fig. 3A), and the resulting construct was transfected into HepG2 cells. As shown in Fig. 3B, promoter activity of deletion mutant increased about 30% compared to wild-type without ATF3 transfection. Furthermore, transfection with the deletion mutant significantly abolished the reduction of P(−294)/Luc promoter activity by ATF3 (Fig. 3B), indicating that the putative ATF/CRE is crucial for the transrepression of AdipoR1 by ATF3.

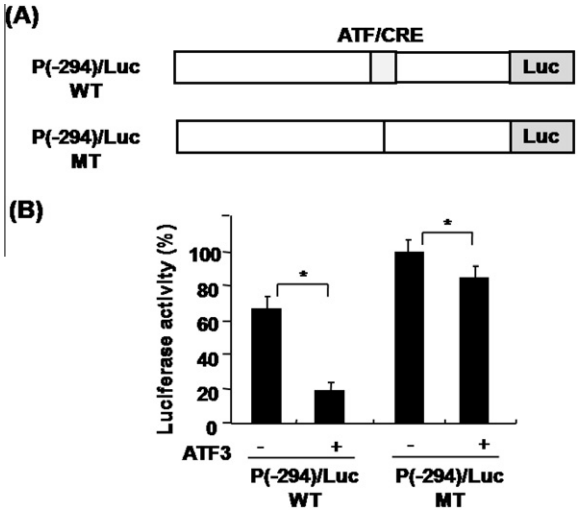

Fig. 3. Confirmation of the biological activity of the ATF3-responsive region. (A) Construction of ATF/CRE-deleted mutant of the human AdipoR1 promoter, P(−294)/Luc. (B) Wild-type or a mutant of the AdipoR1 promoter was transfected into HepG2 cells with ATF3 expression vector and the luciferase activities were measured. Luciferase activities are presented relative to the observable highest activity from the P(−294)/Luc MT. The asterisks indicate a *P* value <0.05 for the bracketed comparisons.

3.4. ATF3 is associated with downregulation of AdipoR1 during hyperglycemia and also with reduction of AdipoR1 by TNF-α treatment in C2C12

Because AdipoR1 expression decreases in hyperglycemic diabetic conditions [13], we analyzed the level of ATF3 expression in C2C12 cells treated with chronic high glucose to determine if ATF3 plays a role in the repression of AdipoR1 expression in skeletal muscle under hyperglycemic conditions. As shown in Fig. 4A (left panel), incubation of C2C12 in 40 mM high glucose for 48 h decreased AdipoR1 expression with an increase in ATF3 expression, suggesting that hyperglycemic-mediated repression of AdipoR1 may be promoted by ATF3. Furthermore, as ATF3 is ER stress-inducible factor, we examined the expression of molecular indicators of ER stress in hyperglycemia-treated C2C12 to see involvement of ER stress in downregulation of AdipoR1 under hyperglycemia. As shown in Fig. 4A (right panel), ER stress proteins including GRP78, phosphorylated-eIF2α increased in hyperglycemia-treated C2C12 cells, suggesting that ER stress induced by hyperglycemia may lead to downregulation of AdipoR1 expression via an increase of ATF3, which leads to insulin resistance partly in skeletal muscle.

Moreover, since recent study reported that TNF-α represses AdipoR1 expression [14], we investigated whether ATF3 may be involved in TNF-α-mediated downregulation of AdipoR1. To this end, we examined expressions of AdipoR1 and ATF3 in TNF-α-treated MIN6N8, C2C12, and 3T3-L1 cells. As shown in Fig. 4B, treatment with TNF-α significantly decreased AdipoR1 expression in the cells, whereas it increased ATF3 expression, suggesting that

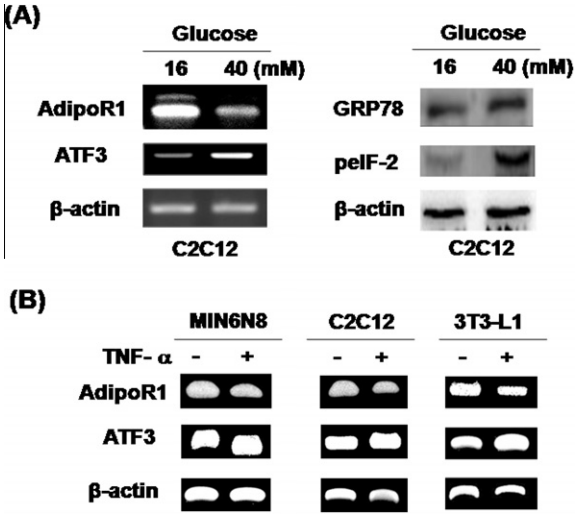

Fig. 4. ATF3 is associated with downregulation of AdipoR1 under hyperglycemia and with reduction of AdipoR1 by TNF-α treatment. (A) Chronic high glucose decreased AdipoR1 expression, but increases ATF3 in C2C12. C2C12 myocyte cells were cultured in 40 mM glucose for 48 h, after which the expression levels of ATF3 and AdipoR1 were determined by RT-PCR using specific primers. ER stress indicator proteins were determined by Western blot. (B) TNF-α decreased AdipoR1 expression, but it increases ATF3 in C2C12. C2C12 myocyte cells were cultured in 20 ng/ml of TNF-α for 24 h, after which the expression levels of ATF3 and AdipoR1 were determined by RT-PCR using specific primers.

suppression of AdipoR1 by TNF-α may be partly mediated through ATF3.

4. Discussion

Adiponectin has recently attracted a great deal of attention due to its antidiabetic and antiatherogenic effects and is expected to be a novel therapeutic tool for diabetes and metabolic syndrome [15]. These effects are considered to be mediated by two related, but distinct receptors for adiponectin, AdipoR1 and AdipoR2, which have recently been cloned and characterized [1]. Interfering with the expression of these receptors impairs the metabolic effects of adiponectin [3]. Therefore, in addition to adiponectin expression and plasma concentration levels, the expression level of the AdipoR1 and AdipoR2 receptors in target tissues may play a role in the control of insulin sensitivity. Actually, the expression of adiponectin receptors was found to be reduced in insulin-resistant ob/ob mice [6,7]. Therefore, adiponectin and adiponectin receptors represent potential versatile therapeutic targets to combat obesity-linked diseases characterized by insulin resistance. Adiponectin itself is controlled under conditions of metabolic stress and by a number of hormones and factors involved in regulation of metabolic function [1,2]. Compared to regulation of adiponectin, which has been studied intensely, regulation of adiponectin receptors is not well understood. It has been reported that the expression of adiponectin receptors was regulated by physiological and pathophysiological states [3–5]; however, the transcription factors involved in regulation of adiponectin receptors have not yet been characterized. Previously, we demonstrated that ER stress-inducible ATF3 negatively regulates AdipoR2 expression in the liver by direct binding onto the promoter region, which may play a role in the development of obesity-induced insulin resistance [11]. In the present study, we investigated whether ATF3 regulates expression of another adiponectin receptor, AdipoR1, to further elucidate the role of ATF3 in adiponectin signaling.

Overexpression of ATF3 using lentivirus decreased AdipoR1 in both liver and skeletal muscle cells. Furthermore, because adiponectin receptors are expressed in pancreatic β cells, we also examined the effect of ATF3 in the pancreatic β cell line, MIN6N8. Lentiviral ATF3 was found to express the expression of adipoR1 in pancreatic β cells. Taken together, ATF3 was found to negatively regulate AdipoR1 expression in both insulin-sensitive cells and insulin secreting cells, suggesting that ATF3 participates in the impairment of adiponectin signaling under insulin resistance or diabetes. To characterize the mechanism of downregulation of AdipoR1 by ATF3, we investigated the promoter activity of the human adipoR1 gene. Both thapsigargin treatment and ATF3 expression efficiently repressed the activities of promoters from −2069 to −294. However, the repression was not found in the −200 promoter, suggesting that the putative ATF3 binding site is located between −294 and −200. A computer-assisted search of the 95-bp region using TFSEARCH revealed two semi-palindromic sequences of ATF/CRE, TGACGCGG (−248/−241 and −231/−224). Chromatin immunoprecipitation showed that ATF3 directly interacted with the AdipoR1 promoter, including the region between −248 and −224. When compared with the ATF3 binding sequence (−86/−94, TGACGCGC) of human AdipoR2 promoter, the sequence in human AdipoR1 promoter was found to be very similar, suggesting that ATF3-mediated repression of both adipoR1 and adipoR2 results from binding of ATF3 to a common binding site on their promoter.

It has been reported that the expression of adiponectin receptors was reduced in hyperglycemic ob/ob mice [6,7]. In addition, incubation of L6 rat skeletal muscle cells with chronic high levels of glucose caused a significant reduction in AdipoR1 expression [13]. In this study, we evaluated ATF3 to determine if it is involved in the hyperglycemia-mediated reduction of AdipoR1. To accomplish this, we determined the level of ATF3 in hyperglycemia-treated C2C12 cells. Hyperglycemia increased the ATF3 level, whereas it decreased AdipoR1 expression, suggesting that ATF3 participates in the reduction of AdipoR1 in skeletal muscle of hyperglycemic conditions. Furthermore, because the AdipoR1 level was also reduced by TNF-α [14], we evaluated the involvement of ATF3 in the reduction of AdipoR1 by proinflammatory cytokine. TNF-α decreased AdipoR1 expression in the C2C12 cells, whereas it increased ATF3 level, suggesting that ATF3 may participate in the reduction of AdipoR1 by TNF-α.

Recently, ER stress has been proposed to be involved in the pathogenesis of diabetes [16]. ER stress induces insulin resistance through suppression of insulin receptor signaling by the hyperactivation of JNK and subsequent serine phosphorylation of insulin receptor substrate-1 (IRS-1) in the liver and adipocytes. Furthermore, ER stress has been shown to cause apoptosis of pancreatic β cells via transcriptional induction of CHOP, activation of JNK, and activation of the ER-localized cysteine protease, caspase-12. Therefore, chronic ER stress is a core mechanism involved in triggering insulin resistance and type 2 diabetes. While ER stress has been widely reported in liver, adipose tissue and pancreas, ER stress in skeletal muscle, the major site of glucose disposal, has not received equal attention. In the present work, we suggest that ER stress may represent the molecular link between hyperglycemia and insulin resistance in skeletal muscle cells. We showed that hyperglycemia induced ER stress as demonstrated by increased expression of GRP78, p-eIF and increased ER stress-inducible ATF3 level in skeletal muscle C2C12 cells in which AdipoR1 expression was decreased, suggesting that hyperglycemia induces ER stress and then decreases AdipoR1 expression via ATF3, which may contribute to insulin resistance in skeletal muscle. Previously, we also demonstrated that ATF3 represses adiponectin expression in adipocytes and AdipoR2 in hepatocyte [11,12]. Together with the current finding that ATF3 downregulates AdipoR1 expression in insulin-sensitive cells, all these results suggest that ER stress may cause attenuation of adiponectin signaling via ATF3 and therefore lead to insulin resistance and type 2 diabetes. This find-

ing strongly indicates that ATF3 plays an important role in ER stress-mediated insulin resistance and type 2 diabetes.

In summary, ATF3 acts as transcriptional repressor in the regulation of AdiopR1, which may impair adiponectin signaling under obesity and diabetic conditions. Therefore, taken together with the previously reported findings that ATF3 negatively regulates the expression of both adiponectin and AdipoR2, ATF3 may contribute to the development of insulin resistance or type 2 diabetes through attenuation of adiponectin signaling.

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