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Hyperglycemia promotes p53-Mdm2 interaction but reduces p53 ubiquitination in RINm5F cells

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Abstract The apoptosis of β cells induced by hyperglycemia has been associated with p53 mobilization to mitochondria and p53 phosphorylation. Murine double minute 2 (Mdm2) induces the degradation of p53 and thereby protects cells from apoptosis. We studied the effect of glucose at high concentration on the ability of Mdm2 to ubiquitinate p53 and promote its degradation. RINm5F cells were grown in RPMI-1640 medium with 5 or 30 mM glucose for varying periods of time. After this treatment, the expression of Mdm2 was measured using real-time PCR. The phosphorylation of Mdm2 at Ser166, p53 at Ser15, and the kinases Akt and ATM were measured by Western blotting. The formation of the p53-Mdm2 complex and p53 ubiquitination was assessed by p53 immunoprecipitation and immunofluorescence. Our results showed that high glucose reduced Mdm2 mRNA expression and protein concentration and increased Mdm2 and Akt phosphorylation, albeit with slower kinetics for Akt. It also promoted p53-Mdm2 complex formation, whereas p53 ubiquitination was suppressed. Furthermore, phosphorylation of both p53 Ser15 and ATM was increased in the presence of 30 mM glucose. These data indicate that high concentration glucose decrease the mRNA expression and cytosolic concentration of Mdm2. However, although the increase in glucose promoted the phosphorylation of Mdm2, it also decreased p53 ubiquitination, thus avoiding p53 degradation. In hyperglycemic conditions, such as diabetes mellitus, the reduction of pancreatic β cells mass is favored by stabilization of p53 in association with low p53 ubiquitination and reduced expression of Mdm2.

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Introduction

Many studies have demonstrated that persistent exposure to high glucose concentrations increases apoptosis and reduces the mass of pancreatic β cells [1, 2]. In addition, postmortem studies have reported that between 40 and 60 % of the mass of β cells is lost even before type 2 diabetes is revealed [3]. However, the mechanisms that activate apoptosis of pancreatic β cells are not fully known. β cell death begins with mitochondrial dysfunction and oxidative stress [4, 5]. Changes in mitochondrial permeability allow the release of pro-apoptotic proteins and the onset of apoptosis [6]. Previous studies have shown that apoptosis in pancreatic β cells induced by hyperglycemia is linked to the translocation of p53 to the mitochondria, as its subsequent phosphorylation in this organelle [7, 8].

Due to the important role of p53 in regulating the cell cycle and activating apoptosis in response to various stress signals (DNA damage, oxidative stress, and metabolic stress), cell survival depends critically on an equilibrium between the synthesis and degradation of this protein [9]. Murine double minute 2 (Mdm2) regulates the activity of p53 through two mechanisms: the proteasome–ubiquitination-dependent degradation of p53 and the repression of p53 transcriptional activity. Mdm2 is an E3 ubiquitin ligase that binds to p53 and attaches ubiquitin molecules, allowing for p53 to be recognized by the proteasome for degradation and protecting cells from apoptosis [10, 11]. Moreover, Mdm2 and p53 participate in a negative feedback loop. Whereas p53 stimulates Mdm2 gene expression, Mdm2 promotes the degradation of p53, decreasing the transcription of Mdm2, closing the feedback loop and allowing p53 levels to increase again [12].

The interaction between p53 and Mdm2 depends on the intracellular environment, which affects the post-translational modification of these proteins [13, 14]. Ser395 Mdm2 phosphorylation by ataxia telangiectasia mutated (ATM) decreases the ability of Mdm2 to promote the degradation of p53. Likewise, ATM phosphorylates p53 on Ser15, which prevents the recognition of p53 by Mdm2 [15]. Regulation of p53 by Mdm2 greatly depends on phosphorylation. Within this context, phosphorylation of Mdm2 on Ser166 and Ser188 by protein kinase B/Akt is thought to be the major regulator of Mdm2 activation because it increases the E3 ubiquitin ligase activity of Mdm2 and the degradation of p53 [16–18]. Furthermore, Akt is an important anti-apoptotic signaling molecule, and thus, Mdm2 phosphorylation may be an alternate route through which Akt protects cells from p53-mediated apoptosis. Additionally, it has been suggested that Akt-induced phosphorylation could favor the entry of Mdm2 into the nucleus [14, 15, 19] and the inhibition of p53 transcriptional activity, which would protect against p53-induced death [14, 18].

Under stress conditions, p53 is important for the activation of a series of pro- and anti-apoptotic genes and is also involved in mitochondrial alterations. Hyperglycemia promotes an increase in the production of reactive oxygen species (ROS) and oxidative stress. In turn, ROS promote the activation of phosphorylation cascades that may interfere with the interaction between p53 and Mdm2. Previous studies have shown that hyperglycemia induces apoptosis in RINm5F cells, which is linked to the translocation of p53 to mitochondria and its phosphorylation in this organelle [7, 8]. Thus, it is likely that hyperglycemia inhibits the interaction of Mdm2 with p53 and/or Mdm2 E3 ubiquitin ligase activity. Any of these situations would prevent p53 degradation and promote its recruitment to the mitochondria, beta-cell dysfunction, and the activation of apoptosis. In this study, we evaluated the formation of the p53-Mdm2 complex and the ubiquitination of p53 in RINm5F cells grown in high concentration glucose.

Materials and methods

Cell culture

RINm5F cells were grown in RPMI-1640 medium (Sigma-Aldrich Co., St. Louis, MO, USA) supplemented with 10 % (v/v) fetal bovine serum (PAA The Cell Culture Company, Pashing, Austria), 2 mM L-glutamine, 1 mM sodium pyruvate (Invitro S.A., Mexico City, Mexico), 23.8 mM sodium bicarbonate, and 20 μg/L gentamycin (GIBCO, Carlsbad, CA, USA) at 37 °C in a humidified atmosphere with 5 % CO_2. At 75 % confluence, cells were detached with 0.025 % trypsin and 2 mM EDTA (Sigma-Aldrich Co., St. Louis, MO, USA) and reseeded in 75-mm^2 flasks at 2.5×10^6 cells per flask in supplemented RPMI-1640 medium. The medium was changed the following day. On the second day, cells were treated with 30 mM glucose (high glucose). Control cells were maintained in 5 mM glucose (low glucose).

Mdm2 expression analysis

Total RNA extraction and cDNA synthesis

Total RNA was obtained from 10^7 cells using TRIzol (Invitrogen, Carlsbad, CA, USA). RNA concentration and purity were measured by assessing the absorbance at A_{260} and A_{280} nm (EPOCH$^®$ system). RNA integrity was confirmed using 1.2 % agarose gels with 0.5 μg/mL ethidium bromide. cDNA synthesis was performed from 5 μg of total RNA with the ImProm II Reverse Transcriptase System kit (Promega, Madison, WI, USA) following the manufacturer's protocols.

Real-time PCR

Mdm2 expression was measured using the primers 5′- AG GAGCGGATCCATGTGCAATACCAACATG-3′ (forward, Tm 59.85 °C) and 5′-CTAGGGGAAATAAGTTAGCA CAAT-3′ (reverse, Tm 59.96 °C) (OligoPerfect^{TM} Designer, Invitrogen, Carlsbad, CA, USA). Real-time PCR (qPCR) was performed using the intercalating fluorophore SYBR Green (which fluoresces upon binding to the minor groove of double-stranded DNA, allowing for quantification of each amplification cycle) with the LC FS DNA Master SYBR Green I kit (Roche Diagnostics GmbH, Mannheim, Germany) following the manufacturer's protocols. The GAPDH primers 5′-GAATGGGAAGCTGGTCATCA-3′ (forward,

Tm 61.02 °C) and 3′-TCTGAGTGGCAGTGATGGTG-5′ (reverse, Tm 60.91 °C) were used as controls.

Cell fractionation

RINm5F cells (2×10^7) were resuspended in 350 µl of buffer A (100 mM HEPES pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.05 mM PMSF, 1M $NaVO_4$, and 10 % IGEPAL), incubated for 10 min at room temperature, and then centrifuged at $16,500 \times g$ for 2 min at 4 °C. The pellet and supernatant were treated as follows: the pellet was resuspended in buffer B (20 mM HEPES, 0.4 mM NaCl, 1 mM EDTA, 1 mM EGTA, 10 % glycerol, 0.5 mM PMSF, and 1M $NaVO_4$) and centrifuged at $15,000 \times g$ for 5 min at 4 °C, and the supernatant was collected as the nuclear fraction. The original supernatant was centrifuged twice at $15,000 \times g$ for 30 min at 4 °C to obtain the cytosolic fraction. An aliquot was used to quantify proteins using the Lowry assay [20], and the lysates were stored at −70 °C until being used for Western blotting.

Western blot analysis of Mdm2, Mdm2 pSer166, Akt pSer473, ATM pSer1981, and p53 pSer15

Proteins (80–100 µg) were separated by SDS-PAGE (10 %) for 1 h at 120 V and then transferred to a PVDF membrane (Immobilon^R-P, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) overnight at 0.8 mA/cm^2 and 4 °C. The transfer was confirmed by 0.1 % Ponceau S red staining solution. Proteins were detected with the corresponding primary antibody (1:1000): anti-Mdm2, anti-pMdm2 Ser166, anti-β-actin, anti-histone H1, anti-Akt, anti-pAkt Ser473, anti-ATM, anti-pATM Ser1981, anti-p53, anti-p-p53 Ser15 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and were incubated overnight at 4 °C. Antibody binding was detected with a horseradish peroxidase-conjugated secondary antibody (MP Biomedicals, Santa Ana, CA, USA) and a chemiluminescence kit (Luminol, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA).

p53 immunoprecipitation

Anti-p53 (Pab 246, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) was added to 500 µg of protein in PBS and incubated for 1 h at 4 °C with orbital shaking. Protein G PLUS-Agarose (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) was then added, followed by orbital shaking overnight at 4 °C. The immunoprecipitated fraction was collected by centrifugation at $1000 \times g$ for 5 min at 4 °C, washed in PBS, resuspended in Laemmli buffer, and separated by SDS-PAGE (10 %) for Western blot analysis

with anti-Mdm2 or anti-ubiquitin at a dilution of 1:1000 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA).

Mdm2 and p53 immunofluorescence

Cells were grown on 8-well chambered coverslips (Thermo Scientific, Rockford, IL, USA) at 25,000 cells/well in the presence of 5 or 30 mM glucose and were analyzed at 24, 48, and 72 h. After treatment, the cells were washed with PBS and fixed for 30 min at 37 °C with 4 % paraformaldehyde. The cells were then washed with PBS-T, permeabilized with 0.3 % Triton-PBS for 15 min, and blocked (Universal Blocking Reagent, Bionex, USA) for 15 min at 37 °C. After blocking, primary antibodies (anti-Mdm2 and anti-p53, 1:10) were added for overnight incubation at 4 °C in a humidified chamber with gentle shaking. Next, cells were washed with PBS-T, and secondary antibodies (anti-mouse IgG Alexa 488 and anti-rabbit IgG Alexa 594, 1:50) were added for a 4-h incubation. Nuclei were then stained with 0.3 mM DRAQ-7 (Biostatus, Leicestershire, UK) by incubating for 20 min at room temperature in the dark. Cells were washed with PBS-T and mounted using VECTASHIELD (Vector Laboratories, San Mateo, CA, USA). The samples were analyzed with a confocal microscope (Carl Zeiss, Axiovert) equipped with an argon/helium/neon laser using the Zen 2009 imaging software (Carl Zeiss, Goettingen, Germany). Image capture was performed with 488 and 543-nm lasers and short pass (BP 505-530 and BP 565-585 for Mdm2 and p53, respectively) or long pass (LP 650 for DRAQ-7) filters. All micrographs were obtained at ×40. The results were analyzed using pseudo-color representations with ImageJ.

Statistical analysis

Analyses were performed using the NCSS statistical software. The results are expressed as the mean ± SD. A multiple-comparison parametric test (ANOVA) was used, and comparisons between groups were assessed with the Tukey–Kramer test and a significance threshold of $p < 0.05$.

Results

The effect of hyperglycemia on Mdm2 expression and concentration

High glucose (30 mM) induced time-dependent reduction in Mdm2 mRNA expression with significant differences from 8 to 72 h compared with the control (low glucose) (Fig. 1a). A similar effect was observed on its protein

concentration, where high glucose significantly reduced Mdm2 levels ($p < 0.05$) in the nuclear fraction at 48 and 72 h. Concomitantly, the amount of Mdm2 in the cytosolic fraction significantly decreased from at 24 to 72 h with high glucose (Fig. 1b; $p < 0.05$).

The effect of hyperglycemia on Mdm2 Ser166 and Akt Ser473 phosphorylation

Mdm2 Ser166 phosphorylation in the cytosolic fraction of RINm5F cells at both 48 and 72 h was significantly higher with high glucose treatment compared with low glucose. In the nuclear fraction, Mdm2 Ser166 phosphorylation was also higher in response to high glucose, but this was only statistically significant at 72 h (Fig. 2a). The elevated glucose concentration decreased Akt phosphorylation in the cytosol during the first 24 and 48 h compared to low glucose, which was followed by a significant increase at 72 h ($p < 0.05$, compared with 24 and 48 h). However, no difference was observed when the 72 h high glucose treatment group was compared with low glucose at 72 h. In the nuclear fraction, a continuous and significant increase in Akt phosphorylation from 24 h ($p < 0.05$) up to 72 h was observed with high glucose (Fig. 2b).

The effect of high concentration glucose on the formation of the p53-Mdm2 complex and ubiquitination

To determine whether the increase in glucose affected the interaction between p53 and Mdm2, we performed an immunoprecipitation assay with an antibody against p53 and Western blot detection of Mdm2. The results showed that in the cytosolic fraction, the treatment of RINm5F cells with 30 mM glucose increased the interaction between p53 and Mdm2 from 24 h ($p < 0.05$) up to 72 h, compared with low glucose. In the nuclear fraction, the p53 and Mdm2 interaction did not change significant after high

glucose treatment compared with low glucose (Fig. 3a). Immunofluorescence and confocal microscopy revealed the presence of p53 and Mdm2 localized together in aggregates in the cytosol, as well as an evident increase in both proteins at 48 and 72 h of treatment, compared with low glucose, supporting the results obtained by Western blot analyses (Fig. 3b).

Because one of the roles of Mdm2 is to ubiquitinate p53, immunoprecipitation of p53 in the cytosolic and nuclear fractions followed by Western blotting with an antibody to ubiquitin were performed. High glucose (30 mM) induced time-dependent reduction in the ubiquitin associated with p53 in relation to the control, in both the cytosolic and nuclear fractions, starting from 4 (cytosol) or 8 h (nucleus) of high glucose treatment with a further reduction to nearly undetectable levels between 24 and 72 h of treatment (Fig. 3c).

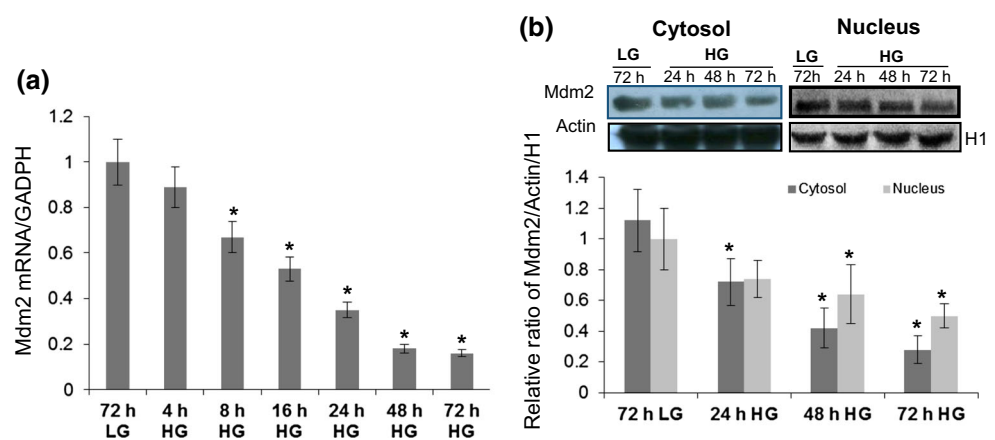## The effect of high glucose on p53 and ATMK phosphorylation

p53 Ser15 phosphorylation in the nuclear fraction increased in the presence of high glucose starting at 24 h and was maintained up to 72 h, despite a decrease in p53 between 24 and 72 h of treatment (Fig. 4). The increase in Ser15 phosphorylation occurred in parallel with the phosphorylation of ATM in the nucleus, which showed a similar pattern starting at 24 h of treatment with high glucose ($p < 0.05$).

Discussion

A reduction in the population of pancreatic beta cells, primarily due to an increase in the rate of apoptosis, precedes the clinical signs of diabetes [3]. The apoptotic death of beta cells due to high glucose concentrations has been linked to p53 translocation to the mitochondria [7, 8],

Fig. 1 The hyperglycemia reduces the expression and concentration of Mdm2. **a** Real-time RT-PCR of Mdm2. *$p < 0.001$ versus 72 h low glucose (LG, 5 mM). **b** Western blot and densitometric analysis of Mdm2. *$p < 0.05$ versus 72 h LG. HG: high glucose, 30 mM. The values represent means and standard deviations from five independent experiments
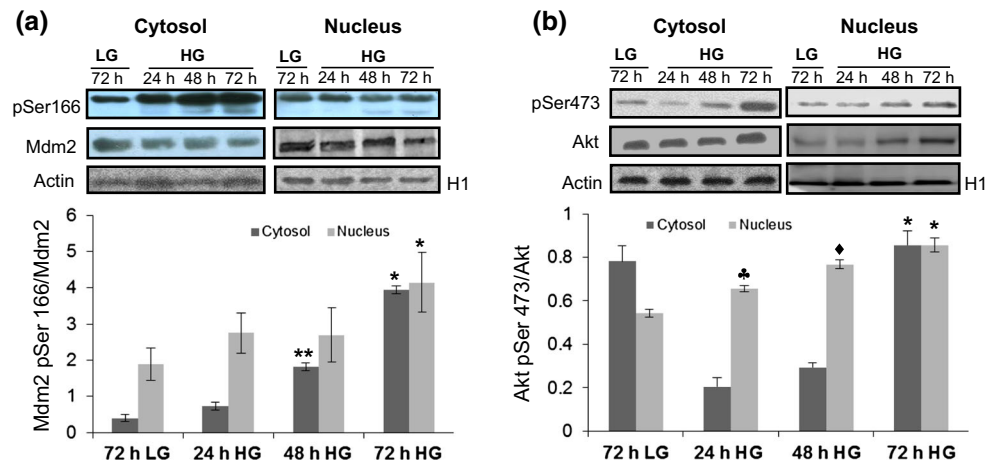
Fig. 2 The effect of high glucose on Mdm2 and Akt phosphorylation in RINm5F cells. **a** Western blot analysis of Mdm2 Ser166 phosphorylation. Cytosol: $p < 0.05$ *versus 72 h low glucose (LG, 5 mM) and 24 h and 48 h high glucose (HG, 30 mM); **versus 72 h LG and 24 h HG. Nucleus: *$p < 0.05$ versus 72 h LG. **b** Akt Ser473 phosphorylation. Cytosol *$p < 0.05$ versus 24 h and 48 h HG.

Nucleus: *$p < 0.05$ versus 72 h LG and 24 h and 48 h HG; *filled diamond* $p < 0.05$ versus 72 h LG and 24 h and 72 h HG; *black club suit* $p < 0.05$ versus 72 h LG and 24 h and 72 h HG. The values represent means and standard deviations from five-independent experiments

decrease interaction of glucokinase with the mitochondrial membrane [6], pro-apoptotic proteins activity and the opening of the permeability transition pore in the inner mitochondrial membrane [7, 21] In addition, the recovery of beta cell populations and the rescue of diabetic phenotype have been shown in p53 knockout mice, highlighting the importance of this protein in the development of diabetes [22, 23].

Although the cytotoxic action of high glucose in the pancreatic β-cells has been attributed to several complementary mechanism, including decreased interaction of glucokinase with the mitochondria [6], Ca^{2+}-dependent increased expression of the transcription factor c-Myc [24] and the Ca^{2+} responsive transactivator [25], and increased oxidative stress [26]; among others, our studies have been focused in the role of p53 as a mediator of high glucose-induced apoptosis and how its action and stability are regulated [7, 8]. In this study, we grew pancreatic RINm5F cells in two glucose concentrations (5 and 30 mM) to determine the effect of hyperglycemia on the ability of Mdm2 to promote the degradation of p53. We found that hyperglycemia decreases the expression of Mdm2 mRNA, as well as cytosolic and nuclear Mdm2 protein levels, but in increases its phosphorylation and the formation of the p53-Mdm2 complex in the cytoplasm. Despite this, p53 ubiquitination was found to be decreased.

The intracellular levels of p53 are determined by its rate of degradation rather than its rate of synthesis. The most common pathway of p53 degradation is the ubiquitin-26S proteasome system [27], which requires ubiquitination of p53 by Mdm2. Mdm2 expression is regulated by p53 [12]. Nonetheless, although it has been shown that p53 is stable

under high glucose concentrations, this protein is translocated to the mitochondria, decreasing its concentration in the cytosol and nucleus [7]; therefore, the expression of Mdm2 induced by p53 is reduced. Furthermore, the hyperglycemia-induced DNA fragmentation previously reported to occur [8, 28] may have affected the expression of Mdm2 mRNA. It is also likely that high glucose concentrations affect the nuclear export signal of Mdm2, promoting its exit from the nucleus and increasing its levels in the cytosol. This would increase the rate of apoptosis because Mdm2 would be hindered from recruiting nuclear p53 and targeting it for degradation.

The interaction between p53 and Mdm2 is regulated by posttranscriptional modifications. The ability of Mdm2 to bind p53 and stimulate its ubiquitination depends on phosphorylation of Mdm2 residues Ser166 and Ser188, which are also linked to the intracellular localization of Mdm2 [29, 30]. In the present study, we did not observe changes in the phosphorylation of Mdm2 Ser166 in the nucleus, but in the cytosol, Ser166 phosphorylation increased with high glucose at 72 h. This result contrasts with previous studies showing that Ser166 phosphorylation in the cytosol is required for Mdm2 nuclear translocation and for the transcriptional inactivation and degradation of p53 [19, 31]. The disruption of Mdm2 phosphorylation interrupts its interaction with p53 and is likely related to the translocation of p53 to other organelles such as the mitochondria and the activation of pancreatic β-cells apoptosis, as previously determined [7, 8].

The involvement of Akt in Mdm2 Ser166 phosphorylation has already been shown [16]. In our study, we observed that Akt phosphorylation, as seen with Mdm2, is
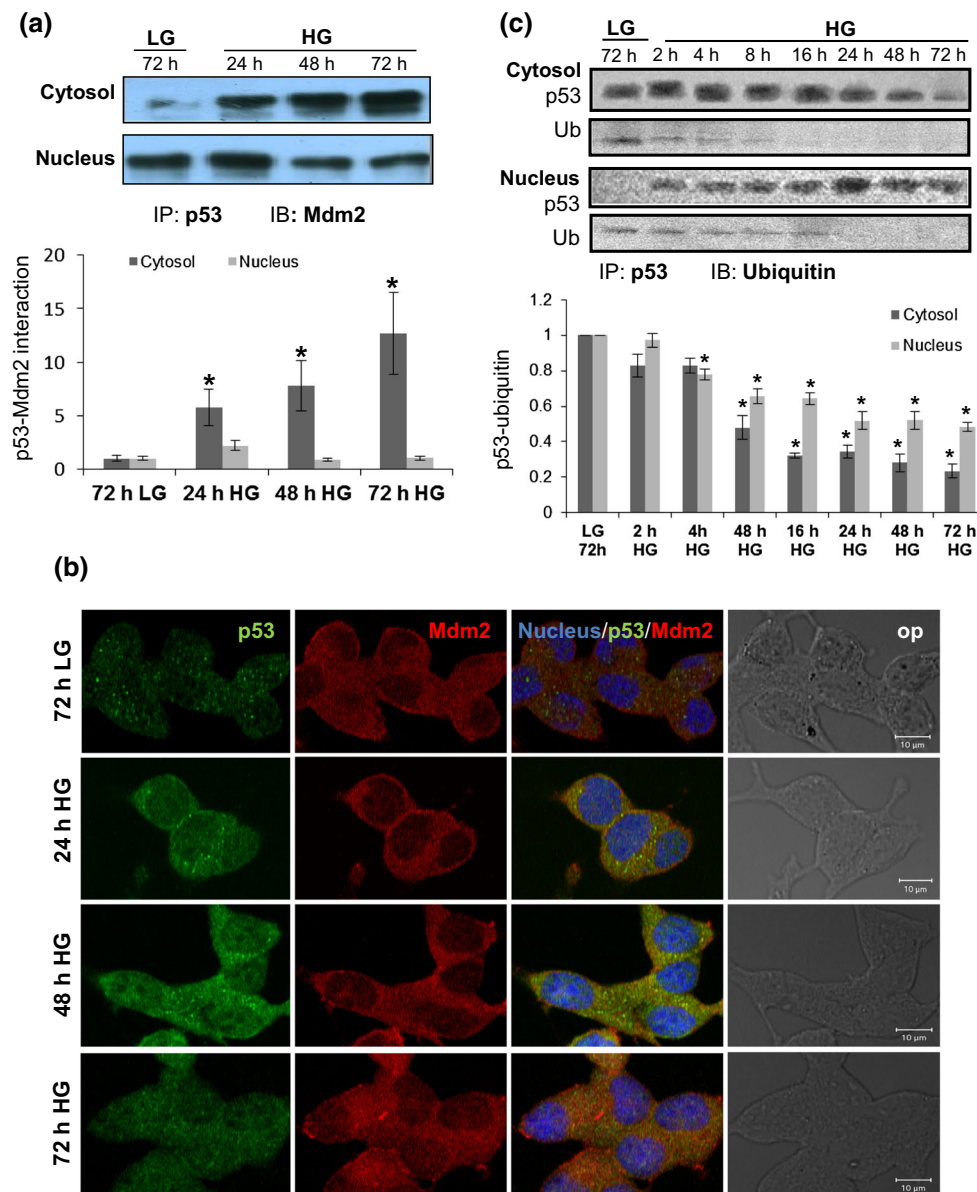
(a)

IP: **p53**   IB: **Mdm2**

(c)

IP: **p53**   IB: **Ubiquitin**

(b)

Fig. 3 The effect of high glucose on p53-Mdm2 association and p53 ubiquitination. Immunoprecipitation was performed with anti-p53, Western blotting with anti-Mdm2 or anti-ubiquitin (Ub). **a** Western blot and densitometric analysis with anti-Mdm2, *$p < 0.05$ versus 72 h low glucose (LG, 5 mM) and **c** Western blot and densitometric analysis with anti-ubiquitin. Cytosol: *$p < 0.05$ versus 8 h, 16 h, 24 h, 48 h, and 72 h high glucose (HG, 30 mM); nucleus: *$p < 0.05$ versus 4 h, 8 h, 16 h, 24 h, 48 h, and 72 h HG. **b** Colocalization of p53 and Mdm2 via immunofluorescence as an indicator of the p53-Mdm2 complex. Nuclei (*blue*), Mdm2 (*red*), p53 (*green*). The values represent means and standard deviations from five independent experiments. (Color figure online)

induced in response to chronic hyperglycemia when apoptosis has already begun [7]. Akt activation promotes the survival of beta cells from diabetic mice [32], and a decrease in Akt activation directly correlates with an increase in hyperglycemia-induced apoptosis [33]. Previously it has been reported that Mdm2 phosphorylation by Akt contributes to p53 regulation by decreasing the transactivation and increasing the ubiquitination of p53 [19, 31]. Furthermore, Mdm2 phosphorylation by Akt inhibits

Mdm2 autoubiquitination and its proteasomal degradation [29, 34]. These studies support a role for Akt in the regulation Mdm2-dependent p53 degradation and the inhibition of high glucose-induced apoptosis. In contrast, our results suggest that formation of the Mdm2-p53 complex is regulated by the phosphorylation of Mdm2 by Akt, but they also indicate that the function of this complex can be regulated by other factors. It should be noted that other kinases may also phosphorylate Mdm2 Ser166, including
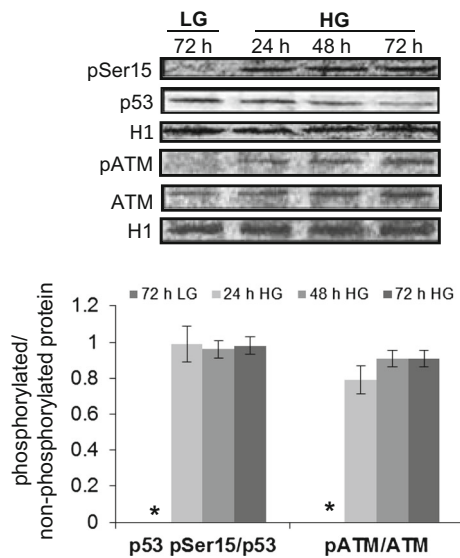
Fig. 4 The effect of high concentration glucose on p53 Ser15 and ATM phosphorylation. Western blot and densitometric analysis of p53 and ATM phosphorylation in RINm5F cells grown in low glucose (LG, 5 mM) or high glucose (HG, 30 mM). $*p < 0.01$ 72 h LG versus 24 h, 48 h, and 72 h HG. The values represent means and standard deviations from five-independent experiments

ERK 1/2 [30]. However, under hyperglycemic states, ERK 1/2 activation decreases and correlates with an increase in RINm5F cells apoptosis [8].

The typical role of Mdm2 is to bind p53, catalyze its ubiquitination, and drive its degradation. In our study, the formation or accumulation of the p53-Mdm2 complex was increased in the cytosol with high glucose treatment, whereas the complex remained almost constant in the nucleus, suggesting that high glucose altered the interaction between these proteins, allowing p53 translocation to the cytosol to initiate apoptosis. These results also indicate that in the cytosol high concentration glucose induces Mdm2 activation and stimulates its interaction with p53. According to our results, low levels of Mdm2 lead to increased cytosolic localization and reduced degradation of p53 [18]. The decreased ubiquitination of p53 observed with high glucose treatment can be explained by the effect of Mdm2 phosphorylation by ATM [35, 36]. Mdm2 Ser395 phosphorylation by ATM has been shown to inhibit Mdm2 E3 ubiquitin ligase activity [15, 37, 38]. An increase in ATM phosphorylation due to hyperglycemia was also seen in our model beginning at 24 h. Another factor that may have affected p53 ubiquitination is a decrease in ATP. Under conditions of hyperglycemia, ATP concentrations are reduced due to an increase in ROS, reduced expression of glucokinase and mitochondrial depolarization [4, 6, 21, 39]. Thus, a decrease in ATP prevents binding between the C-terminal glycine of ubiquitin and the lysine residues of p53.

Phosphorylation of p53 also regulates its interaction with Mdm2. It has been previously shown that high glucose concentrations do not alter p53 transcription or its concentration [7, 8]. Instead, high glucose causes modifications of the intracellular distribution of p53, favors its mitochondrial localization, and promotes its phosphorylation, preventing the degradation and increasing its biological activity [11]. In this study, p53 Ser15 phosphorylation increased with high glucose. Ser15 phosphorylation is necessary for Thr18 phosphorylation, which inhibits p53-Mdm2 binding and p53 degradation [40, 41]. Phosphorylation of p53 Ser15 by ATM has also been shown in cells with DNA damage induced by genotoxic stress [42] and has been linked to an increase in pro-apoptotic genes such as Bax, contributing to the induction of apoptosis in various cell types. Our results show that high concentration glucose promote ATM activation and p53 Ser15 phosphorylation in the nucleus, which is associated with reduced p53 ubiquitination.

In short, our results indicate that reduced expression and cytosolic concentration of Mdm2, as well as decreased ubiquitination of p53 in the cytosol and nucleus, prevent p53 degradation and contribute to the mechanism of apoptosis induction in RINm5F cells under hyperglycemic conditions.

Conflict of interest The authors declare no conflict of interest.

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