The RhoGDI-α/JNK signaling pathway plays a significant role in mycophenolic acid-induced apoptosis in an insulin-secreting cell line

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Mycophenolic acid (MPA)-induced β-cell toxicity is an important factor for islet graft function. The signal transduction mechanisms underlying this process have not been fully explored. Using a proteomics approach, we examined protein expression patterns in MPA-treated β-cell lines and found that RhoGDI-α expression is altered by MPA-treatment. We examined the relationship between RhoGDI-α expression and activated JNK during MPA-induced apoptosis. Cells were treated with N-acetyl-cysteine (NAC), caspase inhibitor, JNK inhibitor, guanosine or GTP for 1 h before being treated with MPA. To investigate the regulatory effects of RhoGDI-α on JNK activity, we examined cells showing either elevated or reduced expression of RhoGDI-α as a result of transfection with cDNA or siRNA constructs, respectively. MPA significantly increased cell death, caspase-3 expression and JNK activation, but it decreased the expression of a protein spot 25 observed by two-dimensional electrophoresis. This protein 25 was identified as RhoGDI-α by mass spectrometry. MPA-induced cell death and down-regulation of RhoGDI-α were prevented by guanosine, GTP or a JNK inhibitor. However, MPA-induced cell death was partially restored by treatment with a caspase inhibitor, but not by NAC treatment. RhoGDI-α expression was not affected by treatment with NAC or caspase inhibitor. Over-expression of RhoGDI-α increased cell viability and decreased activated JNK expression following exposure to MPA, whereas knockdown of RhoGDI-α enhanced MPA-induced cell death and increased the activation of JNK. In conclusion, MPA induces significant apoptosis in insulin-secreting cells via down-regulation of RhoGDI-α linked with increased JNK expression. This RhoGDI-α/JNK pathway might be the focus of therapeutic target for the prevention of MPA-induced islet apoptosis.

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1. Introduction

Guanine nucleotides (GNs) modulate many biochemical reactions, and their cellular concentrations must be maintained at critical levels [1]. The rate-limiting enzyme in the synthesis of new GNs is inosine 5′-monophosphate dehydrogenase (IMPDH), a soluble enzyme that converts IMP into guanosine monophosphate, and subsequently to GNs [2]. Mycophenolic acid (MPA) is a specific IMPDH inhibitor that has been widely utilized as an immunosuppressive drug after transplantation [3]. It effectively induces cell-cycle arrest in late G1 phase in lymphocytes, and it induces differentiation or apoptosis in cultured cells [4–9]. Although MPA has been used to prevent islet graft rejection, its β-cell toxicity may be an important issue for long-term outcomes [10–15]. MPA may have negative effects on β-cell function and viability in vitro [12]. A recent study suggests that MPA-mediated GTP depletion blocks DNA synthesis and induced β-cell death with characteristics of apoptosis [9].

The Rho GTPase family proteins include RhoA, Rac1, and Cdc42, and may have an important role in a variety of cellular process such as cell morphology, motility, proliferation, differentiation, and apoptosis [16,17]. Activation of the Rho and Rac proteins may enhance apoptosis in a variety of cell types [18]. Currently, three human Rho guanine nucleotide dissociation inhibitors (RhoGDIs) have been identified that regulate the nucleotide exchange process by preventing GDP release. These are the ubiquitously expressed RhoGDI-α [19], the hematopoietic cell-selective RhoGDI-δ [20], and RhoGDI-γ [21]. Essmann et al. [22] reported that RhoGDI-δ (D4-GDI) is cleaved and translocated into the nucleus by caspase-3 activation during apoptosis. However, the homologous RhoGDI-α does not undergo similar processing [23]. RhoGDI-α was initially purified

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from rabbit intestine and bovine brain cytosol as an inhibitor of both dissociation of GDP from the Rho protein and of the subsequent binding of GTP to Rho [24,25]. RhogDI-α is over-expressed in multiple types of tumors, such as ovarian, breast, and hepatic cancer, and it may be involved in the regulation of drug-induced apoptosis [24,26]. RhogDI-α seems to have a broad activity and is able to form 1:1 complexes with isoprenylated RhoA, RhoB, Rac1, Rac2, and Cdc42 [25,27–31]. The binding and inhibitory activity of RhogDI towards all of these proteins seems to be similar, at least in vitro [32]. Rac1-mediated apoptosis involves TNF-α-induced ERK1/2, p38 mitogen-activated protein kinase (MAPK) activation, and Akt activation [33]. In addition, RhogDI-α may be involved in a wide variety of cellular functions, including apoptosis and inhibition of GDP dissociation from the Rac1 protein [26,34,35]. As a result of the activity of RhogDI-α, GTPase is maintained in an inactive form, which prevents Rac1 activation by guanine nucleotide exchange factors. These cellular effects mediate apoptosis via the pathway involving c-Jun NH2-terminal kinase (JNK) and p38 MAPK [29,30,33,36]. MAPK activation plays a critical role in MPA-induced cell death in the insulin-secreting cell line, HIT-T15 [37]. The objectives of this study were to identify significant proteins associated with MPA-induced cell death and to examine the potential relationship between RhogDI-α expression and activation of JNK during MPA-induced apoptosis in the insulin-secreting cell line, RIN-5F.

2. Materials and methods

2.1. Cell culture and chemicals

RIN-5F rat islet cells (American Type Culture Collection, Manassas, VA) were cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (Gibco, Grand Island, NY), 2 mM β-glucose, 0.5 unit/ml penicillin, and 100 µg/ml streptomycin at 37 °C in a humidified atmosphere of 5% CO2. Cells at 80% confluence were switched to serum-free medium containing 10 µM MPA in the presence or absence of various experimental reagents.

2.2. Sample preparation for electrophoresis

Unless otherwise stated, chemicals used in this study were purchased from Sigma Chemicals (St. Louis, MO). Cultured cells were washed with 1 M sucrose solution (pH 7.2) and suspended with an appropriate volume of lysis buffer containing 7 M urea, 2 M thiourea, 4% CHAPS (Amresco, Solon, OH), 40 mM Tris–HCl, 100 mM DTT, 2 mM tributyl phosphine, 25 mM NH4HCO3/50% acetonitrile, 100 µg/ml streptomycin at 37 °C in a humidified atmosphere of 5% CO2. Cells at 80% confluence were switched to serum-free medium containing 10 µM MPA in the presence or absence of various experimental reagents.

2.3. Two-dimensional electrophoresis (2-DE) and protein staining

Prior to isoelectric focusing, immobiline DryStrips (pH 4–7) were rehydrated at room temperature for 16 h in a lysis buffer containing 1.2 mg of cellular lysates. Isoelectric focusing was performed at 18 °C with a current limit of 50 µA/strip. Voltage was increased progressively to a total of 120 kV h: 1 h at 100 V, 1 h at 500 V, 2 h at 1000 V, and 10 h at 4000 V until the final voltage was reached. The focusing apparatus was an Etan IGPPhor (Amersham). IGP strips were equilibrated for 15 min by gentle shaking in 6 M urea, 2% SDS, 5 mM tributyl phosphine, 1 M Tris–HCl, and 30% glycerol. Vertical SDS gradient slab gels (8–18%) were used in the second dimension of electrophoresis. The second-dimension gels were overlaid with a solution containing 0.5% agarose, 1 M Tris–HCl, 0.1% SDS, and a trace of bromophenol blue. Electrophoresis was conducted in an Etan Dalt II System tank (Amersham) running at 18 mA per gel at 18 °C. Fixation of proteins was performed by incubating gels in 3% phosphoric acid and 50% methanol for at least 1 h, followed by three washes of 15 min in distilled water. The gels were stained overnight with Coomassie Brilliant Blue G-250 (G250 in 34% ethanol, 3% phosphoric acid).

2.4. In-gel digestion and mass spectrometry

The Coomassie Blue-stained gels were scanned using a densitometer (UMAX PowerLook 2100XI, Dallas, TX) at a resolution of 200 dots per inch in the transparency mode. The gel images were analyzed using the ProteomeWeaver software system (Definiens, Munich, Germany). Statistical comparisons were made using Student’s t-test with statistical significance defined at p<0.05. Comparisons were made between replication groups comprising 3 or 4 gels of sufficient quality per each time period after MPA treatment. Analysis of protein expression profiles in MPA-treated samples was performed for each time point. After normalization of protein spots, intensities for 20, 24, and 28 h were transformed by the log2 value of the ratio between the experimental and control results at that time point. Protein spots of interest were manually excised from the Coomassie-stained 2-DE gels for in-gel digestion. Gel slices were washed with a buffer containing 25 mM NH4HCO3/50% acetonitrile, dried completely with a Speedvac evaporator dryer (BioTRON, Daejeon, Korea), and digested at 37 °C for 18 h with trypsin (10 µg/ml in sequencing grade, 25 mM ammonium bicarbonate). Peptides were solubilized with 60% acetonitrile/0.1% trifluoroacetic acid (TFA), and desalted with an in-house column packed with C18 porous beads. The bound peptides were eluted in 0.6 µl of elution buffer (1 mg/ml α-cyano 4-hydroxy-cinnamic acid solution in 60% acetonitrile/0.1% TFA) and spotted onto a MALDI plate (Applied Biosystems, Foster City, CA). Peptides mixtures were analyzed with a 4700 Proteomics Analyzer (Applied Biosystems), and peptide masses were matched against the theoretical peptide masses of all proteins in the National Center for Biotechnology Information (NCBI) or SWISS-PROT databases.

2.5. MTT reduction assay

Cell survival was measured by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) reduction assay at the indicated time periods following drug treatment. Approximately 2×104 cells from each stable cell line were plated on a 25 mg/ml poly-o-lysine-coated 48-well plate (Corning, NY), cultured in RPMI 1640 for 2 days, and switched to serum-free medium with or without 10 µM MPA. For experimental purposes, N-acetyl-cysteine (NAC, 1.0 mM), a caspase inhibitor (z-VAD-fmk, 200 µM) or a selective JNK inhibitor (SP600125, 15 µM) [38] was administered 1 h before MPA treatment. The rate of cell survival was measured using the colorimetric MTT reduction assay [39]. After MTT solution was added to cells to a final concentration of 1 mg/ml, the cells were incubated for 30 min at 37 °C, and lysed in 20% SDS in 50% aqueous dimethylformamide (DMF) for 20 h, 24 h, or 28 h. The optical density of the dissolved formazan grains was assessed at 540 nm using an ELISA plate reader (Molecular Devices, Palo Alto, CA). Measurements from each treatment were expressed as a percentage of survival relative to the corresponding matching control.

2.6. Western blot analysis

To determine expression levels of RhogDI-α, the cleaved form of caspase-3, activated JNK, and MKK4/7, cultured RIN-5F cells were treated with experimental reagents for the indicated times and the expression level of each protein was measured by western blot analysis. Briefly, cultured cells were washed with ice-cold PBS containing 2 mM EDTA and lysed on ice for 10 min in a buffer containing 50 mM Tris–HCl,
2 mM EDTA, 1.0% Triton X-100, and protease inhibitor cocktail. Lysates were centrifuged at 12,000 g for 15 min at 4 °C after trituration on ice through a syringe fitted with a 26 gauge needle. Total protein concentration was measured using the Bio-Rad protein assay kit (Bio-Rad, Hercules, CA). Aliquots of 20 µg of each sample were mixed with loading buffer (60 mM Tris–HCl, 25% glycerol, 2% SDS, 14.4 mM 2-ME, 0.1% bromophenol blue), separated on 12.5% SDS-PAGE gels, and transferred to a nitrocellulose membrane. Membranes were blocked for 1 h with 5% non-fat dry milk and incubated with antibodies against RhoGDI-α, JNK, MKK4/7, or caspase-3 (Cell Signaling, Beverly, MA). Blots were washed and then incubated for 1 h at room temperature with horseradish peroxidase (HRP)-conjugated anti-rabbit and anti-goat antibody. Sites of antibody binding were visualized by enhanced chemiluminescence (ECL; Amersham) western blotting detection system, and quantified using a densitometer (Bio-Rad).

2.7. Annexin V and propidium iodide staining

Apoptosis and cell nuclear damage were assessed by Annexin V-FITC and propidium iodide (PI) staining, and analyzed with fluorescent microscopy. Cells were grown in 6-well plates and treated with 10 µM MPA. Both attached and floating cells were processed using the ApoAlert Annexin V-FITC apoptosis kit (Clontech, Palo Alto, CA) according to the manufacturer’s instructions. Apoptosis and nuclear cell damage were examined by fluorescence microscopy and fluorescence-activated cell sorting (FACS; Becton Dickinson, Sunnyvale, CA).

2.8. RhoGDI-α cloning

In vitro RhoGDI-α transcription templates were amplified by PCR from RIN-5F cDNA using the following primers: sense primer, 5′-CTAGAGCTGAGCTAGCTGACCAAACTGAA-3′, and antisense primer, 5′-CAGCTGGAGCTCAGTAGTCCTTCCATTCC-3′. The PCR products were cloned into mammalian and bacterial expression vectors with flanking restriction sites engineered into the PCR oligonucleotides. After subcloning the cDNA into pAcGFP1-C2 vector, the insert was fully sequenced by the dideoxy nucleotide chain termination method using Taq DNA polymerase. The vector was introduced into insulin-secreting cells by a 2 h transfection using 4 µl of Lipofectamine 2000 (Invitrogen, Carlsbad, CA) in 200 µl of serum-free culture medium. RIN-5F cells (1.6×10⁶) cultured in 6-well plates were transiently transfected either with small interfering RNAs (siRNAs) targeting RhoGDI-α or with scrambled negative control siRNA (mock control). All siRNAs were purchased from BIONEER Inc. (Daejeon, Korea). The following RhoGDI-α sequences were used: sense, 5′-GAGAUCGUGUCCGGCAUGA(dTdT)-3′, and antisense, 5′-UCAGUCGGACACGACUCUC(dTdT)-3′. Transfection into RIN-5F cells was performed using the Lipofectamine 2000 Reagent (Gibco). Transfected cells were analyzed by immunoblotting approximately 18 h after transfection.

2.9. Statistical analysis

Data are given as mean±standard error. Significance of differences was determined by one-way ANOVA and post hoc Student’s t test. Statistical significance was set at a p value less than 0.01 or 0.05.

3. Results

3.1. Effect of MPA on cell death and caspase-3 activation in RIN-5F cells

To examine the effect of MPA on an insulin-secreting cell line and determine its apoptotic mechanism, RIN-5F cells were treated with MPA. MPA induced cell death in a time-dependent manner. Approximately 50% of cells died between 20 and 24 h following treatment with 10 µM MPA (Fig. 1A). Expression of cleaved caspase-3 was higher in MPA-treated cells than in mock-treated cells. MPA-induced caspase-3 activation was inhibited by the addition of 100 µM GTP between 20 and 28 h (Fig. 1B). The addition of MPA significantly increased the number of Annexin V- and PI-stained cells compared with the control (Fig. 1C).

![Fig. 1](image-url)
3.2. Proteomics analysis to detect altered protein expression following MPA treatment

To identify downstream mediators whose expression is affected by MPA treatment and analyze the functional role of these changes in expression, we used a proteomics analysis. The protein expression profile was determined with 2-DE. More than 860 matched protein spots ranging from approximately 10 to 100 kDa were detected in the RIN-5F cells treated with or without MPA at the indicated times. Expressions of 32 spots (numbered 1–32) changed by at least 1.5 fold at 20, 24 and 28 h after MPA treatment (Supplementary Fig. 1). These spots were subjected to in-gel digestion with trypsin, MALDI-TOF mass spectrometry, and a database search using either the NCBI or SWISS-PROT database. Supplementary Table 1 summarizes the proteins identified in MPA-treated RIN-5F cells and provides their accession number, molecular weight/isoelectronic point (pI), peptides matches, MOWSE score [40], and sequence coverage of the peptide analysis.

3.3. Identification of RhoGDI-α as a novel target

Among the 32 proteins, we focused on spot 25, which showed an exact match to RhoGDI-α with MALDI-TOF mass spectrometry (accession number: gi|56541074). Expression levels of this protein decreased at 20, 24, and 28 h after MPA treatment (Fig. 2A). Western blot confirmed that MPA decreased expression levels of RhoGDI-α (Fig. 2B).

3.4. Effects of pre-treatment with guanosine, GTP, NAC or a caspase inhibitor on MPA-induced cell death and expression levels of RhoGDI-α

To investigate the molecular mechanisms of MPA-induced cell death and the expression of RhoGDI-α, RIN-5F cells were pre-treated 1 h before MPA treatment with 500 µM guanosine, 100 µM GTP, 1.0 mM NAC, or 200 µM z-VAD-fmk. Guanosine and GTP effectively prevented MPA-induced cell death and blocked the down-regulation of RhoGDI-α expression (Fig. 3). Cell death induced by MPA was partially restored by the caspase inhibitor z-VAD-fmk, but not by the anti-oxidant NAC (Fig. 3A). NAC and z-VAD-fmk did not affect expression levels of RhoGDI-α in RIN-5F cells treated with MPA (Fig. 3B).

3.5. The inhibition of JNK activity is related to RhoGDI-α expression

As RhoGDI-α expression levels in MPA-treated cells were not affected by NAC or caspase inhibitor, we speculated the significant role of RhoGDI-α apart from caspase activity or interaction with reactive oxygen species in MPA-induced apoptosis. To test our hypothesis, we investigated the relationship between the RhoGDI-α expression and the MAPK pathway. MPA increased the levels of activated JNK and

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**Fig. 2.** Proteomics analysis of protein expression altered by MPA treatment and identification of RhoGDI-α. (A) The serial magnification view of the interesting spot (spot 25) was excised, destained, and enzymatically digested as described in Materials and methods. The peptide masses were matched with the theoretical peptide masses obtained from the NCBI database (RhoGDI-α in Supplementary Fig. 1 and Table 1). Quantification of spot 25 protein expression over time was presented with arbitrary units (fold intensity relative to the untreated control) from four independent experiments. *p < 0.01 versus control. (B) The MPA-induced change in RhoGDI-α expression was confirmed by western blot analysis. Western blots are representative of three independent experiments. Quantification of blot over time was presented with arbitrary units (fold intensity relative to the untreated control) from three independent experiments. *p < 0.01 versus control.
MKK4/7 in selected time courses (Fig. 4A), but pre-treatment with GTP reduced these levels (Fig. 4B). These findings suggested that MPA-induced cell death might occur through an increase in the levels of activated JNK and MKK4/7. The potential regulatory effects of JNK on RhoGDI-α expression were therefore investigated to evaluate whether changes in RhoGDI-α expression were associated with JNK and MKK4/7 activation. The selective JNK inhibitor SP600125 (15 µM) was administered to cells 1 h prior to MPA treatment. Viability of cells treated with both MPA and the JNK inhibitor was 22% greater than that of cells treated with MPA alone at 28 h (Fig. 4C). Furthermore, pre-treatment with JNK inhibitor could prevent the reduction in RhoGDI-α expression caused by MPA (Fig. 4D). These results suggest that inhibition of JNK activity affects the expression level of RhoGDI-α during MPA-induced apoptosis in insulin-secreting cells.

3.6. Increased expression of RhoGDI-α confers resistance to apoptosis

To investigate the potential modulatory effects of RhoGDI-α on the JNK pathway, cell lines that either over- or under-expressed RhoGDI-α were generated. Western blot analysis revealed that RhoGDI-α levels were higher in cDNA-transfected cells than in mock-transfected controls (Fig. 5A). RhoGDI-α expression in transfected cells remained similar to control cells under normal growth conditions, and cells appeared morphologically similar to the control cells (data not shown). Over-expression of RhoGDI-α prevented MPA-induced cell death successfully, while RhoGDI-α knockdown accelerated cell death following MPA treatment (Fig. 5A). Expression levels of activated JNK and MKK4/7 were increased in a RhoGDI-α knockdown cell line, but not in the over-expressed cell line, at 28 h after MPA treatment (Fig. 5B). These results suggest that expression levels of RhoGDI-α may affect activation of JNK.

4. Discussion

Previous reports have shown that a small GTPase can trigger signals that activate the MAPK pathway and thereby lead to apoptosis in different cell types [18,33,41]. Similarly MPA can induce cell cycle arrest and activate the MAPK pathway in cultured cells, leading to apoptosis [9,37]. Recently, small GTPases have been shown to be regulated by binding to inhibitory RhoGDI molecules. Among them, RhoGDI-α has been shown to be over-expressed in many tumor types and has been implicated in the regulation of apoptotic signals [26,42]. However, until now, there has been no solid evidence suggesting a relationship between the RhoGDI and MAPK pathways in MPA-induced apoptosis. Although the effect of MPA on RhoGDI expression has not been extensively investigated, a previous study showed that other drugs such as busulfan can reduce expression of RhoGDI-α in some cell types [34]. We speculated that altered RhoGDI-α expression could affect both MPA-induced cell cycle regulation and apoptosis through the JNK pathway.

First we confirmed that MPA induces caspase-dependent apoptosis in RIN-5F cells (Fig. 1). Mass spectrometry and 2-DE were used to investigate the expression levels of certain key regulator proteins associated with MPA-induced apoptosis. Among the 32 proteins whose expression changed following MPA treatment (Supplementary Fig. 1 and Supplementary Table 1), spot 25 protein identified as RhoGDI-α was examined for its potential association with the MPA-induced apoptotic pathway. In western blot analysis, RhoGDI-α expression in RIN-5F cells was found to be down-regulated at 20, 24, and 28 h after MPA treatment (Fig. 2). We investigated the role of RhoGDI-α on MPA-induced apoptosis by NAC or caspase inhibitor pre-treatment (Fig. 3). Previous reports have suggested that altered RhoGDI-α expression during the apoptotic process may contribute to formation of reactive oxygen species (ROS) or caspase activation [24,26,43]. However, in the present study, MPA-induced cell death was partially recovered by pre-treatment with a caspase inhibitor, but not with ROS inhibitor (Fig. 3A). Our results are partly consistent with those of Takebe et al. [44], who suggested that MPA-induced cell death was dependent upon caspase activity, but was less likely to be associated with ROS in a multiple myeloma cell line. Furthermore, in our study, RhoGDI-α expression levels in MPA-treated RIN-5F cells were not significantly affected by NAC or caspase inhibitor (Fig. 3B). These findings led us to speculate that, rather than directly affecting caspase activity or interacting with ROS, RhoGDI-α might play another role in MPA-induced apoptosis.

To test our hypothesis, we investigated the relationship between the RhoGDI-α expression and the MAPK pathway. RIN-5F cells treated with a JNK inhibitor showed higher cell viability and RhoGDI-α
Fig. 4. Increase in levels of activated JNK/MKK 4/7 expression in relation to RhoGDI-α. Cells were treated with 10 µM MPA alone or with 100 µM GTP or JNK inhibitor (SP600125) for the time periods indicated. (A) Cell lysates (20 µg) were loaded onto gels and subjected to immunoblot analysis using antibodies against activated forms of MKK4/7 and p-JNK. The lower JNK blot confirms equal loading in each lane. MPA increased the levels of activated MKK4/7 and p-JNK. Quantification data are expressed as arbitrary units of intensity relative to the control value of JNK from three separate experiments while only representative western blots are shown. *p<0.01 versus control. (B) Cell lysates (20 µg) were separated by SDS-PAGE and immunolabeled with monoclonal antibodies against MKK4/7 or p-JNK. β-actin and JNK exposure levels are shown to verify similar loading. Pre-treatment with GTP inhibited the activation of JNK and MKK4/7. Quantification data are expressed as arbitrary units of intensity relative to the control value of β-actin or JNK from three separate experiments while only representative western blots are shown. *p<0.01 versus control. (C) Cell viability was measured by the MTT assay. RIN-5F cells treated with MPA and SP600125 showed significantly higher cell viability than cells treated with MPA alone. Each point represents mean±standard error in triplicate. **p<0.01 versus control. (D) Cell lysates (20 µg) were separated by SDS-PAGE following a 28 h treatment with both MPA and the JNK inhibitor SP600125. Immunoblot analysis was performed to detect p-JNK and RhoGDI-α. The normal expression level of RhoGDI-α was observed when cells were pre-treated with SP600125. Quantification data are expressed as arbitrary units of intensity relative to the control value of β-actin from three separate experiments while only representative western blots are shown. *p<0.01 versus control.
expression than cells treated with MPA alone (Fig. 4). These results suggest that inhibition of JNK activation can affect RhoGDI-α expression. In addition, we observed that over-expression of RhoGDI-α prevents MPA-induced cell death and decreases expression levels of activated JNK and MKK4/7, whereas knockdown of RhoGDI-α enhances MPA-induced cell death and increases the levels of activated JNK and MKK4/7 (Fig. 5). Although we demonstrated a close link between RhoGDI-α expression and activated JNK activity, the link between RhoGDI-α and MPA-induced islet apoptosis needs to be further evaluated to better understand how the JNK pathway is involved. Several studies have indicated that RhoGDI-α may negatively regulate the activities of small G proteins of the Rho family by shutting off their GDP/GTP cycling. This cycling allows Rho family proteins to function as molecular switches that regulate downstream signal transduction processes [45–47]. RhoGDI-α shows a broad range of activities towards several Rho family proteins in vivo, including Rac1, Rac2, and RhoA [48]. For example, down-regulation of RhoGDI-α leads to activation of Rac1 [49]. Rac1 activation, in turn, leads to activation of JNK and p38 MAPK, which may affect apoptotic cell death [33,50]. Until now, the link between RhoGDI-α and the MAPK family
in MPA-induced cell death has not been fully understood, either in vivo or in vitro. To our knowledge, this is the first study to demonstrate that RhoGDI-α expression levels can affect JNK activation and vice versa during MPA-induced islet cell death. Based on our findings, we may propose dual signaling cascades (RhoGDI-α/JNK and conventional caspase pathway) to explain the MPA-induced apoptotic mechanism in RIN-5F cell lines (Fig. 6). However, specific small GTPase and upstream JNK kinase besides MKK4/7 for MPA-induced apoptosis require further investigation in the near future.

In conclusion, MPA treatment induces significant apoptosis in insulin-secreting cells by down-regulating the expression of RhoGDI-α, which is closely linked with activated JNK. This novel pathway is not directly related to caspase or reactive oxygen species. How the interaction between RhoGDI-α and the JNK pathway mediates MPA-induced islet apoptosis requires further evaluation. The RhoGDI-α/JNK signaling pathway may be a suitable therapeutic target for preventing MPA-induced cell death in insulin-secreting cells.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cellsig.2008.11.009.

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