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Effect of GAPDS overexpression on high glucose-induced oxidative damage

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ABSTRACT

The occurrence of infertility in diabetic patients is attributed to oxidative damage of peroxidized products. High glucose-induced mitochondrial oxidative stress and glycolytic enzyme inactivation is considered to be an important mediator for sperm dysfunction. In this study, we successfully constructed TM3-GAPDS stable strain and investigated the role of sperm specific glyceraldehyde-3-phosphate dehydrogenase (GAPDS) on high glucose-induced apoptosis in TM3 cells. High glucose decreased the protein expression of SOD2 and catalase, while the level of intracellular ROS and the apoptosis - related protein increased in TM3 cells. Furthermore, high glucose-induced oxidative stress and apoptosis were reversed by GAPDS overexpression or antioxidant treatment. In conclusion, our data suggest that GAPDS overexpression antagonize high glucose-induced apoptosis by controlling ROS accumulation in TM3 cells.

Diabetes is attracting more and more attention due to its rapidly increasing prevalence and society in general [1]. The International Diabetes Federation estimated that there were 410 million people living with diabetes [2]. Diabetes can induce long-term damages, dysfunctions and failures of various organs. Cumulative evidence suggests that diabetes mellitus (DM) can lead to male reproductive dysfunction by affecting spermatogenesis and sperm motility as well as increasing sperm apoptosis [3,4].

Type 2 DM (T2DM) accounts for the vast majority (90–95%) of all DM cases [5,6] and is also associated with a high prevalence of subfertility/infertility and 35% of T2DM are reported to be infertile. A recent study reported that hundreds of infertile couples with diabetes possess lower volume of ejaculates, sperm count and percentage of motile sperm as compared to healthy of couples with proven fertility [7]. Data from animal models suggest that prediabetes also impairs several male reproductive parameters due to altered testicular metabolism and mitochondrial bioenergetics. Furthermore, prediabetic animals present increased testicular oxidative stress (OS), although the mechanism involved are poorly understood.

Glycolysis enzymes are localized in the midpiece and principal piece region of the flagellum and is the major source of ATP required for sperm flagellar motility, capacitation and fertilization [8,9]. Several studies have suggested that the reactive oxygen species (ROS) can result in inactivation of glycolytic enzyme and reduce sperm motility. Several glycolytic enzymes (GEs), including hexokinase isoenzyme1(HK1S), phosphoglycerate kinase (PGK2), Sperm specific glyceraldehyde-3-phosphate dehydrogenase (GAPDS) and pyruvate kinase (PKS), have been identified and reported to play a key role in ATP production of sperm cells [10]. GAPDS is a spermatozoa specific glyceraldehyde-3- phosphate dehydrogenase, located in the principle of the sperm tail and is closely related to the movement of sperms. Previous study has reported that the reduction or inactivation of GAPDS activity will block the progressive motility of spermatozoa and is the main cause for the reduction of the fertility. Several studies have suggested that the reactive oxygen species (ROS) is overproduced in diabetics and the activity of sperm specific glyceraldehyde-3-phosphate dehydrogenase (GAPDS) are easily oxidized in the presence of ROS [11,12].

In the present study, a stable strain was constructed by lentivirus technology and a series of cell tests were carried out with the GAPDS stable strain as a carrier. We further evaluated the effect of

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GAPDS overexpression on the expressions of apoptosis-related genes and on the ROS levels in the TM3 cell line.

1. Materials and methods

1.1. Cell line and reagents

The TM3 cells was preservation by our lab. pBABE-Puro vector plasmid were purchased from Beina company (Shanghai, China). N-acetylcysteine (NAC) and D-glucose were obtained from Sigma (St. Louis, MO). Antibodies for GAPDS, superoxide dismutase 2(SOD2), Catalase were purchased from Abcam (Cambridge, UK). Antibodies for Bax and Bcl-2 were purchased from Santa Cruz (Calif., USA.). TUNEL Apoptosis Detection Kit, qPCR Master Mix and Transfection Reagent were obtained from Promega (Madison, WI).

1.2. Cell culture and treatment

TM3 cells were cultured in RPMI-1640 medium (Gibco, Carlsbad, Calif., USA) supplemented with 10% fetal bovine serum (Beijing TransGen Biotechnology Co. Ltd., China) and 1% penicillin/streptomycin (Beijing TransGen Biotechnology Co.) in 37 °C incubator with a humid atmosphere of 5% CO 2. When grown to 60%–80% confluence, the cells were stimulated with normal concentration D-glucose medium (NG, 5.6 mmol/L), high concentration medium (HG, 30 mmol/L), NAC plus HG medium (HG + N) at indicated time points. A total of $1.0*10^6$ cells were seeded into each well of 6 well plates.

1.3. Construction of the stable cell line TM3-GAPDS

Human GAPDS gene was cloned into pBABE-puro vector. The pBABE-GAPDS or pBABE-puro empty vector was transfected into 293T cells with Lipofectamine 2000 (Invitrogen, Carlsbad, Calif., USA). After 48 h, the supernatant was harvested and concentrated. Subsequently, TM3 cells were infected with pBABE-GAPDS or pBABE-puro lentivirus. The stable control and overexpressed GAPDS cell lines were screened by the limited dilution method, confirmed by semiquantitative PCR and Western blotting.

1.4. Protein extraction and western blot

Whole cell protein was extracted using RIPA lysis buffer containing protease and phosphatase inhibitor. 50 mg protein were separated on 10%–12% SDS-PAGE gels and then transferred to PVDF membranes by electrophoresis. The membranes were incubated overnight with the indicated primary antibodies. The protein bands were visualized with corresponding horseradish peroxidase-conjugated secondary antibodies. The densitometry of the protein bands was measured using ImageJ software.

1.5. Intracellular ROS detection

The TM3 cells were cultured in 6-well plates and treated as previously indicated, then the cells were washed three times with PBS. Next, the cells were incubated with 10 mM/L fluorescence probe DCHF-DA in PBS at 37 °C for 30 min and washed in order to remove the residual probes, trypsinized and suspended in PBS. The intracellular ROS was measured with a flow cytometer (BD Immunocytometry Systems, Franklin Lakes, NJ). The results were analyzed by FlowJo software.

2. Results

2.1. The construction of TM3-GAPDS stable cell lines

Human GAPDS gene was amplified by RT-PCR technique from epididymis tissues. Then the gene was cloned into a pBABE lentiviral vector. The recombined lentiviral vector pBABE-GAPDS or pBABE empty vector was transfected into 293T cells with Lipofectamine2000 (Invitrogen, Carlsbad, Calif., USA). Semi-quantitative PCR and Western blotting suggested that the expression of GAPDS was remarkably increased in TM3-GAPDS cells compared with untreated TM3 cells lines (Fig. 1). These results demonstrate that a stable cell line TM3-GAPDS was successfully constructed.

2.2. Overexpression of GAPDS protects TM3 cells from oxidative injury

To confirm whether overexpression of GAPDS could resist HG-induced oxidative stress in TM3 cells, we examined the expression of superoxide dismutase 2, catalase and the level of ROS in cells. As shown in Fig. 2A and B, the expression of SOD2 and catalase protein decreased by 36.5% and 64.6% respectively in the HG group compared with the control group. While there were no obviously changes in GAPDS + HG group and GAPDS group (Fig. 2). Next we quantified the intracellular ROS levels. As shown in Fig. 3, HG caused a 2-fold increase of ROS levels in the cells of HG group compared with the negative control group (NG) (Fig. 3A and B). Whereas GAPDS overexpression (HG + GAPDS) and NAC (HG + N) treatment could significantly reverse HG induced ROS accumulation (Fig. 3D and E). As a transfection control, the TM3 cells transfected pBABE-vector plasmid (HG + control) had no effect on high glucose induced oxidative stress (Fig. 3C).

2.3. Overexpression of GAPDS protects TM3 cells from apoptosis induced by high glucose

The involvement of GAPDH in HG-induced apoptosis has previously been demonstrated. However, whether GAPDS is involved in HG-induced apoptosis in reproductive system remains unclear. As shown in https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5819916/figure/f7-ijmm-41-03-1608/ Fig. 4A,B, there were no obvious changes in the expression of apoptosis-related proteins in the high glucose pre-cultured 12 h, while the ratio of Bax/Bcl-2 and the expression of Fas-L, markers of cell apoptosis were markedly increased following HG exposure. The ratio of Bax/Bcl-2 and the expression of Fas-L were increased 3.84-fold, 2.08-fold respectively after high glucose incubation for 48 h. whereas treatment with GAPDS significantly suppressed the HG-induced increase of apoptosis markers. Next, we examined whether overexpression of GAPDS could reverse HG-induced apoptosis in TM3 cells.

As shown in https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5819916/figure/f7-ijmm-41-03-1608/ Fig. 4 C,D, the significant increase of Fas-L, GRP78 and Bax/Bcl-2 ratio could be reduced by overexpression of GAPDS for 56.25%, 24%, 43% respectively in GAPDS + HG group compared with HG group.

3. Discussion

The purpose of this study is to determine the role of GAPDS in the process of oxidative damage and apoptosis induced by diabetes. First we successfully constructed TM3-GAPDS stable strain and identified by western blot and PCR. We investigated the role of sperm specific glyceraldehyde-3-phosphate dehydrogenase (GAPDS) on high glucose-induced apoptosis in TM3 cells. The

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