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Original Paper

Peroxyredoxin 6 Protects RIN-M5F Pancreatic Beta Cells Against Streptozotocin-Induced Senescence

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Key Words
Streptozotocin • β-cells • RIN-m5F • ROS • Peroxiredixin 6 • H2AX histone • p21 • HSP90-β • HSP90-α

Abstract
Background/Aims: There are evidences that a decrease in the functional activity of pancreatic β-cells under type 2 diabetes conditions may be associated with their senescence, therefore, senotherapy may be a prospective strategy for the diabetes treatment. ***Methods:*** The senotherapeutic potential of peroxiredoxin 6 (PRDX6) was studied in RIN-m5F pancreatic β-cells with streptozotocin-induced senescence by measuring markers, associated with senescence. ***Results:*** Exposure to streptozotocin (STZ) resulted in the senescence of the β-cells. The addition of PRDX6 to the culture medium of RIN-m5F β-cells before treatment with STZ decreased the levels of the following senescence markers: the percentage of SA-β-Gal-positive cells, the phosphorylation of histone H2AX and p21 proteins, and the secretion of the proinflammatory cytokine IL-6 but not the anti-inflammatory cytokine IL-10. These effects were accompanied by a decrease in the production of reactive oxygen species (ROS) and the restoration of impaired NF-κB activation. In addition, PRDX6 altered the production of the heat shock protein HSP90: the production of the constitutive form of HSP90-beta decreased, while the level of inducible HSP90-alpha increased. ***Conclusion:*** PRDX6 prevented the senescence of RIN-m5F cells in response to the DNA damage-inducing agent streptozotocin, indicating a potential protective role of PRDX6 in type 2 diabetes mellitus.

Introduction

Epidemiological studies of diabetes mellitus are regularly conducted by the World Health Organization (WHO) and the International Diabetes Federation (IDF). In 1998, researchers

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predicted that the number of patients with type 1 and type 2 diabetes would increase from 135 million in 1995 to 300 million by 2025 [1]. As of the end of 2021, 537 million patients were suffering from type 1 and 2 diabetes, and according to forecasts, this number will reach 783 million in 2045 [2]. The present study aimed to address an important medical and biological problem associated with treating type 2 diabetes mellitus (T2D). Among all of the cases of diabetes mellitus that are diagnosed every year, more than 90% of patients suffer from type 2 diabetes [3, 4]. A main symptom of both types of diabetes is an increase in the concentration of glucose in the blood. Although type 1 diabetes (T1D) is insulin-dependent, associated with the destruction of pancreatic β-cells, and patients must take insulin for their whole life, T2D patients do not require lifelong insulin administration. Furthermore, their pancreatic β-cells only partially lose their functional activity [5], as shown by the loss of their secretory function and a decrease in β-cell mass [6].

Type 1 diabetes (T1D), which accounts for approximately 5% of all diabetes cases [7], occurs when β cells are selectively destroyed by an autoimmune process. The development of autoimmune diabetes is preceded by the infiltration of pancreatic islets by immune cells. Ultimately, the breakdown of self-antigen tolerance allows autoreactive T cells to become activated and attack β-cells, resulting in a loss of insulin secretion. However, innate immune cells, such as macrophages and dendritic cells (DCs), are the first cells to invade islets during insulitis [8–10].

More than three decades ago, it was shown that glutathione peroxidase activity and peroxide resistance are approximately 20 times greater in the liver and kidneys than in the pancreas [11]. This important finding indicates the natural vulnerability of pancreatic β-cells to oxidative stress. Hyperglycemia leads to the production of reactive oxygen species (ROS) and reduces the effectiveness of endogenous antioxidant defense systems in diabetes mellitus [12]. The antioxidant defense mechanisms include both enzymatic and nonenzymatic systems. There is convincing evidence of oxidative stress in both type 1 and type 2 diabetes, which is accompanied by the death and senescence of β-cells [13]. Furthermore, increased production of reactive oxygen or nitrogen species may deplete endogenous antioxidant enzymes (superoxide dismutase, glutathione peroxidase and catalase) and decrease the concentration of some low molecular weight antioxidants, such as vitamin D [14]. A decrease in the activity of peroxiredoxins under conditions of oxidative and carbonyl stress may be an important factor triggering the molecular mechanisms of oxidative damage in the vascular wall in atherosclerosis and diabetes mellitus [15, 16]. Accounting for the reduced activity of endogenous antioxidant enzymes, more attention has been given to the correction of pathology by the use of exogenous administration of natural protein preparations, such as PRDX6, which has antioxidant activity and can protect pancreatic β-cells during the development of diabetes.

We believe that the main reason for the decrease in the functional activity of pancreatic β-cells under T2D conditions may be associated with their aging or senescence. Oxidative stress is often accompanied by DNA damage, leading to a so-called DNA damage response, manifested as proliferation arrest and the expression of senescence markers. These cells do not die but partially retain their functions, though they are dysregulated in some ways. Additionally, these cells acquire a senescence-associated secretory phenotype (SASP), which is characterized by the production of proinflammatory mediators, among others, that aggravate oxidative stress. We propose that such a shift in β-cells may account for some features of type 2 diabetes.

In the present study, we used a T2D cell model. This involved culturing rat insulinoma β-cells (RIN-m5F) in the presence of streptozotocin (STZ), a known inducer of both insulin-dependent and non-insulin-dependent diabetes mellitus in animals. STZ selectively affects pancreatic β-cells through the glucose transporter GLUT2, triggering DNA alkylation and the generation of reactive oxygen and nitrogen species, leading to changes in pancreatic β-cell DNA, including DNA fragmentation. STZ-induced DNA damage activates poly-ADP ribosylation. This process leads to the depletion of cellular NAD+, a further decrease in ATP content and subsequent inhibition of insulin synthesis and secretion [17] and provokes cell senescence [18].

Previously, we showed that the natural antioxidant protein PRDX6 has a wide range of biological activities, one of which is a pronounced senomorphic effect. When administered exogenously, this protein can neutralize ROS during genotoxic stress caused by ionizing radiation, both in the extracellular environment and inside the cell. This is due to its ability to undergo intracellular translocation resulting from phospholipase activity and modulation of TLR4/NF-κB signaling [19, 20]. We were the first to show that PRDX6 is an agonist of the TLR4 receptor complex [19], which regulates insulin homeostasis and β-cell viability [21]. In this vein, PRDX6 appears to be an attractive agent in senotherapy for antidiabetic therapy.

Notably, we studied the mechanisms underlying the senoprotective effect of exogenous PRDX6 using 3T3 fibroblasts. Moreover, we demonstrated that PRDX6 penetrates 3T3 fibroblasts, which is important for potential biomedical applications [22]. More recent work has assessed the role of peroxiredoxin 6 in neutralizing X-ray-induced oxidative stress and its effect on gene expression, the preservation of radiosensitive tissues and postirradiation survival in animals [23]. In the mentioned work, the effective dose of PRDX6 was assessed.

In the present work, the mechanisms by which PRDX6 affects cell senescence were investigated via the accumulation of senescence markers (SA-β-Gal, H2AX, and p21). In addition, the effect of PRDX6 on inflammatory and stress response levels was assessed by measuring the synthesis of proinflammatory cytokines; the transcription factor NF-κB; and the heat shock proteins HSP70, HSP90β and HSP90α in RIN-m5F cells. PRDX6 may exert a senomorphic effect on RIN-m5F β-cells, indicating the possibility of reducing the pathological consequences of type 2 diabetes mellitus.

Materials and Methods

Culture of RIN-m5F β-cells

RIN-m5F cells were grown in culture flasks in a medium consisting of a mixture of RPMI/DMEM (PanEco, Russia) at a 1:1 ratio (total glucose content 8.0 mM) supplemented with 10% fetal calf serum and a mixture of antibiotics and antimycotics (Merck, Germany) in accordance with the manufacturer's recommendations at 37 °C and 5% CO_2. Mycoplasma testing has been carried out for the cell line. After 3-7 passages, the cells were used in the experiments. Streptozotocin (Sigma–Aldrich, USA) was added to the culture medium at a final concentration of 4 mM. Peroxiredoxin 6 was obtained using genetically engineered constructs that were subsequently used to transform *E. coli* BL21 (DE3) cells [24]. Peroxiredoxin 6 at a concentration of 150 μg/ml was added together with or 1 hour before STZ. Within each independent experiment, the characteristics of the samples were measured in parallel in 9-12 repetitions, and an average value was obtained. The average values from four experiments were used to determine the significance of differences between groups (n=4). Cells that were not subjected to additional treatment with STZ and/or PRDX6 were used as controls.

Measurement of oxidative stress in RIN-m5F β-cells

The level of oxidative stress was measured using the diacetyl derivative of 5, 6-carboxy-2',7'-dichlorodihydrofluorescein (carboxy-H2DCFDA) (Invitrogen, USA). Carboxy-H2DCFDA is a chemical analog of fluorescein and is commonly used as an indicator of oxidative stress in cells. This nonfluorescent molecule is readily converted to the green fluorescent molecule form (carboxy-DCF) after the intracellular removal of acetate groups by intracellular esterases. DCFH (and carboxy-H2DCFDA) do not react with superoxide, hydrogen peroxide, or nitric oxide. The DCFH/carboxy-H2DCFDA ratio reflects peroxidase-catalyzed oxidation. RIN-m5F cells were precultured for 24 h in a 96-well plate (2.5×10^4 cells per well in 100 μl of DMEM), washed with PBS, and filled with a freshly prepared solution of carboxy-H2DCFDA (Invitrogen, USA) in sterile DMSO at a concentration of 2.5 μM in DMEM supplemented with 2% FTS. RIN-m5F cells were incubated with carboxy-H2DCFDA in the dark for 1 hour. With the addition of carboxy-H2DCFDA, other reagents were added. Cells treated with carboxy-H2DCFDA without other agents were used as controls. Fluorescence was measured using an Infinite 200 fluorescence plate reader (Tecan, Austria) at an excitation wavelength of 480 nm and an absorption wavelength of 530 nm (Ex/Em = 485/535 nm). Three independent experiments were performed (three repetitions for each experimental group), with nine technical replicates in each group (n = 9).

Measurement of survival (cytotoxicity test)

RIN-m5F cells ($2x10^4$ cells in 0.1 ml per well) were cultured in 96-well plates in RPMI 1640 medium supplemented with 10% fetal calf serum, 0.2% L-glutamine and a mixture of antibiotics and antimycotics at 37 °C and 5% CO_2. After 24 hours of incubation, 1 μg/ml actinomycin D (Sigma–Aldrich, USA) was added to the formed monolayer of RIN-m5F β-cells, and PRDX6 and/or STZ were then added. After 24 hours of incubation, the washed monolayer was stained for 10 minutes with a 0.05% solution of crystal violet (Sigma–Aldrich, USA), the wells were thoroughly washed with tap water, and 100 μl of 1% sodium dodecyl sulfate was added to each well. After 10 min, the optical density was measured at 546 nm using a plate spectrophotometer (Multiscan EX; Thermo Electron Corporation, USA). Cell viability (%) was calculated using control samples as a reference.

Cytokine production measurement

Twenty-four hours after the addition of STZ and/or peroxiredoxin 6, the RIN-m5F cells were removed from the 24-well plates and centrifuged for 5 min at 2000 rpm, after which the supernatants were collected and used for immediate analysis. For ELISA, we used primary rabbit antibodies against mouse IL-6, IL-10, or TNFα (all from Affinity Biosciences, China); secondary goat antibodies against rabbit immunoglobulins IgG, IgA, and IgM conjugated with biotin (Imtek, Russia); and horseradish peroxidase conjugated with staphylococcal streptavidin (Imtek, Russia). The cells were incubated with antibodies according to the manufacturer's recommendations. Each stage of the ELISA was accompanied by washing the wells three times with a PBS solution containing 0.05% Tween 20. To visualize binding, the TMB substrate for horseradish peroxidase was used in an aqueous solution of 0.1 M sodium citrate (pH 5.4) containing a source of hydrogen peroxide (Imtek, Russia), and the reaction was stopped by adding an equal volume of 5% sulfuric acid. Optical density was measured at 450 nm using a microplate spectrophotometer (Multiscan EX; Thermo Electron Corporation, USA).

Assessment of the senescent RIN-m5F cell count

The senotherapeutic effect of the preliminary application of PRDX6 was studied by adding PRDX6 to the cell culture medium 1 hour before the addition of STZ. Additionally, the protective effect of the combination of STZ and PRDX6 was studied. The cells were cultured for 120 hours at 37 °C and 5% CO_2 and then fixed, and SA-β-Gal activity was determined by the intensity of X-gal staining, as described previously [25]. The samples were examined and photographed using an Axio Imager M1 light scanning microscope (Carl Zeiss, Germany). The number of senescent cells was assessed by the percentage of SA-β-Gal-positive cells using the ImageJ program (NIH, USA).

SDS–PAGE electrophoresis and immunoblotting

To prepare protein samples from RIN-m5F β-cells, the cell suspension was washed out of the culture medium with 0.9% (w/v) NaCl and subjected to three freeze–thaw cycles. Before the freeze–thaw cycles, protease and phosphatase inhibitors (100×) were added to the suspension according to the manufacturer's recommendations (Thermo Fisher Scientific, USA). Solubilization buffer for electrophoresis and β-mercaptoethanol (2–5%) were then added, and the samples were boiled for 5 min. The protein concentration was measured using a NanoDrop2000 spectrophotometer (NanoDrop, USA). Protein samples (10 μl) were added to each well for 10% (w/v) Ds-Na-PAGE. The presence of proteins in the samples was determined by Western blot analysis using the following sets of primary antibodies: rabbit antibodies against phH2AX (AF3187, Affinity, China), p21 (AF6290, Affinity, China), HSP90α (BF0084, Affinity, China), HSP90β (BF0215, Affinity, China), total NF-κB (total-NF-κB) (AF5006, Affinity, China), NF-κB phosphorylated at Ser536 (ph-NF-κB (Ser536)) (AF2006, Affinity, China), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (PAB932Mu01, Cloud-Clone Corp, China). Goat antibodies conjugated to rabbit immunoglobulins IgG, IgA, and IgM with horseradish peroxidase (P-GAR, Imtek, Russia) were used as secondary antibodies. An enhanced chemiluminescence (ECL) system (Bio-Rad, Germany) was used to identify proteins. Images of the blots were acquired using a TFX 35.WL transilluminator (Vilber Lourmat, France). Proteins were quantified by densitometry using the Qapa program (ver. 3.7, Russia). Three independent experiments were carried out (cells from different passages were used) for each protein. The resulting digital data were normalized to the corresponding loading control (GAPDH bands) and are expressed in relative units.

Statistical analysis

Statistical analysis was performed using the Statistica 6.0 software package (StatSoft, USA). To determine the significance of differences, one-way analysis of variance (ANOVA) with Tukey's post hoc test was used. Differences were considered significant at $p < 0.05$. The Shapiro–Wilk test was used to check the normality of the distribution. The W value was not significant in any case ($p > 0.05$).

Results

PRDX6 reduces STZ-induced senescence of RIN-m5F cells

Streptozotocin is particularly toxic to insulin-producing β-cells of the mammalian pancreas. The genotoxic effect of this agent led to increased senescence of these cells. Our data showed that the presence of STZ in the RIN-m5F culture medium led to an increase in the number of senescent cells in the culture (Fig. 1a, 1b) and the accumulation of the senescence markers ph-H2AX and p21 (Fig. 2).

In the presence of PRDX6, the percentage of SA-β-Gal-positive cells decreased (Fig. 1b), as did the levels of the senescence marker p21 protein and the phosphorylated form of histone H2AX (Fig. 2), provided that PRDX6 was added to the culture medium 1 h before STZ treatment.

Our results indicated that PRDX6 has a senescence-preventive effect on RIN-m5F rat insulinoma β-cells. The preliminary addition of PRDX6 prevented STZ-induced senescence of β-cells, as assessed by the number of SA-β-Gal-positive cells, and the levels of the senescence markers p21 and phH2AX also decreased. This finding indicates a pronounced senotherapeutic effect of the antioxidant protein PRDX6 and confirms the senomorphic activity of this protein that we previously discovered (22, 26) in relation to the pathological consequences of T1D.

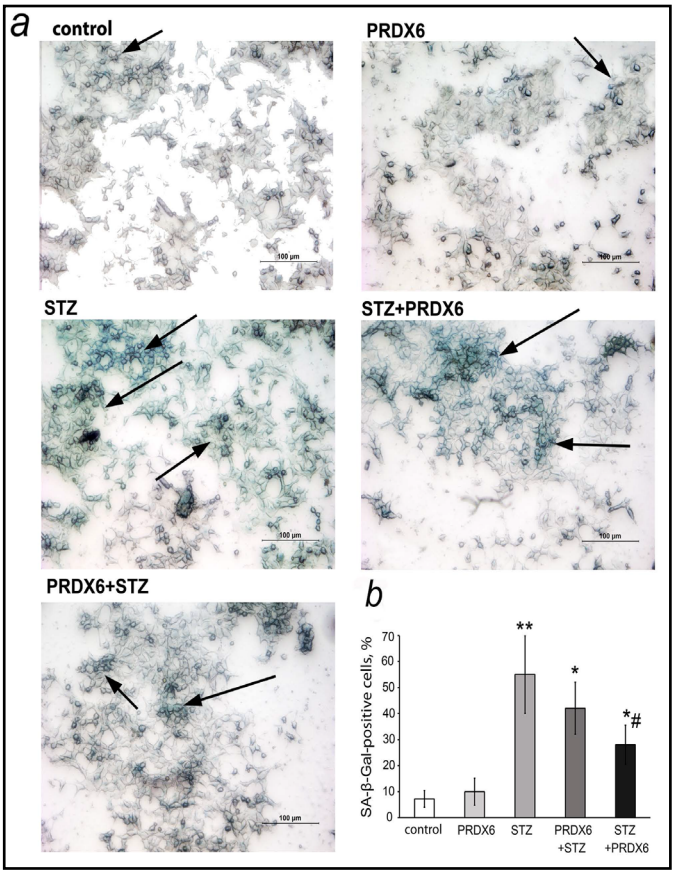

Fig. 1. Effect of streptozotocin and peroxiredoxin 6 on the accumulation of the senescence marker SA-β-Gal in RIN-m5F cells. Panel a: SA-β-Gal-positive cells (green staining) in groups without drug treatment (control), 150 μg/ml peroxiredoxin 6 (PRDX6), 4 mM streptozotocin (STZ), peroxiredoxin 6 plus streptozotocin simultaneously (STZ +PRDX6) and peroxiredoxin 6 one hour before streptozotocin (PRDX6+STZ). Arrows on microphotographs indicate groups of stained senescent cells. Panel b: Percentage of SA-β-Gal-positive cells of the total number of cells in the field of view according to image processing data. Counting was carried out in 4-5 fields of view, and the average value and standard deviation were calculated. *- significant difference from the control group ($p<0.05$), **- significant difference from the control group ($p<0.01$), # - significant difference from the STZ group ($p<0.05$)

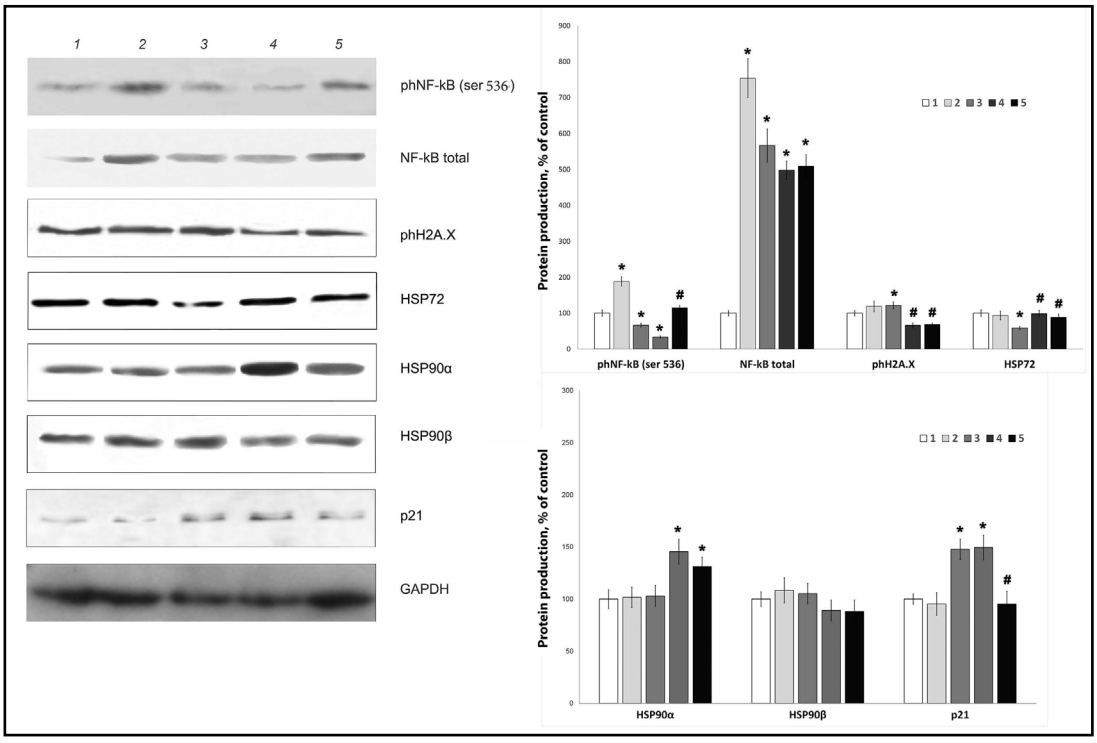

Fig. 2. Effect of streptozotocin and peroxiredoxin 6 on the production of senescence-related proteins in RIN-m5F cells. Representative images of protein bands obtained by Western blotting and densitometry are shown. 1 - control, 2 - PRDX6, 3 - STZ, 4 - STZ+PRDX6 simultaneously, 5 - PRDX6 one hour before STZ. The values are the means of 3 independent experiments ± SEMs normalized to the protein loading control (GAPDH) and to the control group. * - significant difference from the control group ($p<0.05$), # - significant difference from the STZ group ($p<0.05$)

Peroxiredoxin 6 normalizes the oxidative stress level and proinflammatory cytokine production in streptozotocin-treated RIN-m5F β-cells

Our data showed that the addition of STZ to the culture medium of RIN-m5F β-cells stimulated the production of ROS and proinflammatory cytokines, supporting the oxidative stress-inducing effect of STZ in β-cells (Figs. 3, 4). The main sign of the protective effect of antioxidants is a decrease in the production of ROS in cells, and this decrease was demonstrated in our experiments (Fig. 3).

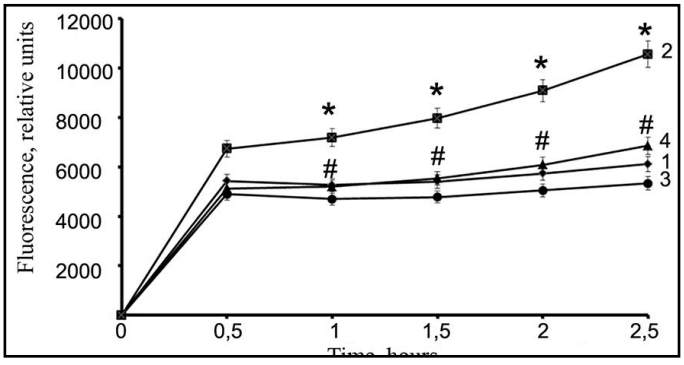

Fig. 3. Effects of streptozotocin and peroxiredoxin 6 on ROS production in RIN-m5F cells. Changes in the fluorescence intensity of the carboxy-H2DCFDA probe were observed at an excitation wavelength of 480 nm and an absorption wavelength of 530 nm upon the addition of STZ and PRDX6. 1 - control, 2 - STZ, 3 - PRDX6, 4 - PRDX6 and STZ. The values are the means of 3 independent experiments ± SEMs. * - significant difference from the control group ($p<0.05$), # - significant difference from the STZ group ($p<0.05$)

STZ increased the concentration of the proinflammatory cytokine IL-6 but not the anti-inflammatory cytokine IL-10. PRDX6 decreased the level of the proinflammatory cytokine IL-6 but not the anti-inflammatory cytokine IL-10 (Fig. 4).

However, when PRDX6 was combined with STZ, the protective effect of the antioxidant was less significant than when it was added to the culture medium 1 hour before STZ, but this effect was only observed for the proinflammatory cytokine IL-6. The activation of the anti-inflammatory cytokine IL-10 increased with the simultaneous addition of STZ and PRDX6 to the cell culture medium and remained significantly increased in the presence of PRDX6 compared with that in the control group.

Effects of PRDX6 and STZ on NF-κB pathway activation and heat shock protein production in RIN-m5F β-cells

When culturing RIN-m5F cells in the presence of STZ, we detected a decrease in the production of the protective inducible heat shock protein HSP70 but not HSP90 (Fig. 2). Moreover, modification of NF-κB signaling was observed: along with a strong increase in the total p65 protein content, its phosphorylation at serine 536 was significantly reduced. The addition of peroxiredoxin 6 to β-cells resulted in the activation of the NF-κB signaling pathway but not heat shock stress proteins. By studying the mechanisms of the senotherapeutic action of PRDX6, we found that the presence of this protein may reduce the aggressive effect of STZ, activating the expression of the stress-inducible heat shock proteins HSP90α and HSP70 but not the constitutive form of HSP90β. In addition, preliminary administration of the antioxidant protein prevented STZ-induced inhibition of p65 phosphorylation at serine 536.

Peroxiredoxin improves the viability of RIN-m5F β-cells in STZ-containing medium

In addition, we assessed the number of live RIN-m5F β-cells after culture with STZ and showed that, under these conditions, the number of these cells was significantly reduced. However, when PRDX6 was present in the culture medium of β-cells exposed to STZ, a significant increase in the number of live RIN-m5F β-cells was observed compared to that in the STZ group (Fig. 5).

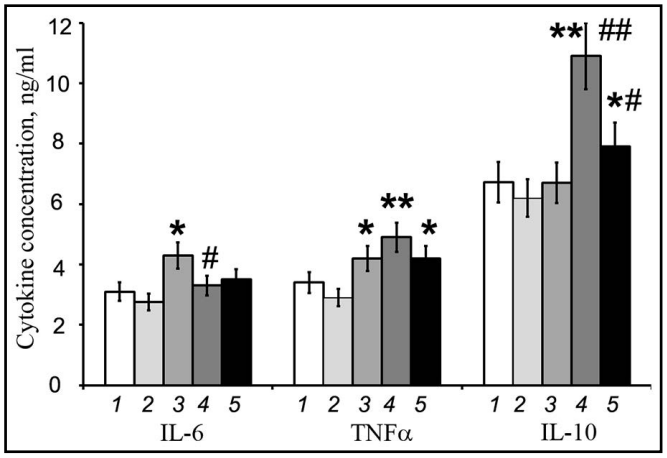

Fig. 4. Effect of streptozotocin and peroxiredoxin 6 on the production of the proinflammatory cytokines IL-6 and TNF-α and the anti-inflammatory cytokine IL-10 by RIN-m5F cells. The results of the ELISA analysis are presented. 1 - control, 2 - PRDX6, 3 - STZ, 4 - STZ-induced PRDX6 simultaneously, 5 - PRDX6 one hour before STZ. The values are the means of 3 independent experiments ± SEMs. *- significant difference from the control group (p<0.05), **- significant difference from the control group (p<0.01), # - significant difference from the STZ group (p<0.05), ## - significant difference from the STZ group (p <0.01)

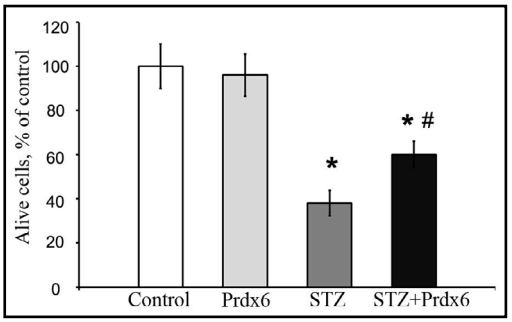

Fig. 5. Effect of streptozotocin and peroxiredoxin 6 on the viability of RIN-m5F cells after 24 hours of incubation. Data on the percentage of viable cells are presented. The number of live cells in the control sample was taken as 100%. Groups: 1 - control, 2 - PRDX6, 3 - STZ, 4 - simultaneous treatment with PRDX6 and STZ. The values are the means of 3 independent experiments ± SEMs. *- significant difference from the control group (p<0.05), # - significant difference from the STZ group (p<0.05), *# - significant difference from the STZ group (p<0.01).

Discussion

Due to the increase in the life expectancy and well-being of humans, metabolic processes associated with aging and age-associated diseases, such as obesity and type 2 diabetes, are currently a serious and socially significant concern. We previously established that PRDX6 exerts a protective effect against the development of type 1 diabetes mellitus as well as against ionizing radiation [22, 26]. However, increased insulin resistance and impaired insulin secretion are important characteristics of type 2 diabetes mellitus and may be associated with, among other factors, age-associated and stress-associated senescence of pancreatic insulin-producing cells. Targeting senescent cells may present many therapeutic opportunities, especially in the context of age-related diseases. This concept has stimulated the testing of senolytic and senomorphic drugs as a new therapeutic paradigm. The emergence of senescent β-cells leads to increased insulin resistance and impaired insulin secretion, which plays a central role in the pathophysiology of TD2 [27, 28].

Age-related changes in β-cell function and proliferation suggest the importance of senescent pancreatic β-cells in the pathogenesis of TD2. Cellular aging or senescence is a condition that develops as a result of replicative shortening of telomeres or the influence of genotoxic, oncogenic and oxidative stresses and is characterized by irreversible cell cycle arrest and cell dysfunctions. Senescent cells are characterized by a set of transcriptional, epigenetic, morphological and metabolic changes and produce a complex secretome (known as the senescence-associated secretory phenotype (SASP)), which causes senescence of surrounding cells (28, 29). Moreover, senescent cells may partially retain their main function, but they may be dysregulated in some aspects, accounting for major internal metabolic rearrangements occurring in these cells. Although many of the detrimental effects of aging can be explained by the SASP, direct evidence for the role of the aggressive phenotype is often lacking given the heterogeneity of the SASP and its context-dependent consequences. The nonspecific immune and heat shock stress protein (HSP) signaling pathways play important roles in cell senescence. These pathways are involved in the regulation of senescent cell survival and the development of the SASP and therefore may be targets for targeted senotherapy for age-associated diseases.

In our model of β-cell senescence, rat insulinoma RIN-m5F cells in the presence of the senescence-inducing agent STZ exhibited all the following signs of cell senescence: an increase in the level of oxidative stress, the percentage of SA-β-Gal-positive cells, the accumulation of the genotoxic stress marker phH2A and the universal inhibitor of the cellular p21 cycle, as well as an increase in the secretion of the proinflammatory cytokine IL-6 and impaired NF-κB phosphorylation. In addition, a decrease in the number of viable cells was observed when the cells were cultured with STZ. Therefore, STZ may be used for the development of cellular models of type 1 diabetes, which is well known, and also for the development of cellular models of type 2 diabetes, accounting for the abovementioned relationships between β-cell senescence and insulin resistance [27, 28].

In view of the role of oxidative stress in the induction of senescence, the aggressive effects of the SASP [29], and the overall vulnerability of β-cells to such harmful influences, the antioxidant enzyme peroxiredoxin 6, which has previously shown senomorphic effects, was used as a serotherapeutic agent in this model. Our data demonstrated that PRDX6, especially when added 1 hour before STZ, was able to prevent many of the pathological effects of STZ on RIN-m5F β-cells and decrease the number of senescent cells, indicating the potential protective role of PRDX6 in type 2 diabetes mellitus.

Identification of reliable markers of aging *in vivo* and models that allow for targeted manipulation of SASP factors inside senescent cells is an urgent task of modern cytogerontology. Therefore, when developing senotherapy for a specific age-associated disease, it is necessary to understand the characteristics of senescence of different types of cells and the possibility of reducing their aggressive secretome since the elimination of certain types of senescent cells can be fatal for living organisms [30-37]. Thus, β-cell

senescence and the development of both type 1 and type 2 diabetes mellitus may have a common genetic basis. Indeed, diabetes mellitus has been reported to coexist with LMNA-mediated progeria and Werner syndrome [38, 39].

Targeting senescent cells to slow the aging process and reduce their level of dysfunction, known as "senotherapy," is gaining momentum. The little-studied area associated with the clinical use of senotherapeutic drugs requires accelerated development. Further research into the molecular mechanisms of cellular senescence in diabetes and the clinical efficacy and safety of senolytic compounds should enable clinical application of this concept.

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Data availability

The data generated in the present study may be requested from the corresponding author

Authors' contributions

Concept and management – E.G. Novoselova; planning and performing of experiments – O.V. Glushkova, M.O. Khrenov, T.V. Novoselova, S.B. Parfenyuk, S.M. Lunin. M.G. Sharapov, Discussion of the results – C.M. Lunin, O.V. Glushkova, M.G. Sharapov. Text writing – E.G. Novoselova. Text editing - S.M. Lunin, O.V. Glushkova, S.B. Parfenyuk, M.O. Khrenov.

Disclosure Statement

The authors declare that they have no competing interests.

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