Redox Biology 14 (2018) 609–617

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Resveratrol attenuates testicular apoptosis in type 1 diabetic mice: Role of Akt-mediated Nrf2 activation and p62-dependent Keap1 degradation

Yuguang Zhao[a], Wenjing Song[a], Zhenyu Wang[b], Zongqiang Wang[c], Xing Jin[d], Jiancheng Xu[e], Ling Bai[a], Yuying Li[a], Jiuwei Cui[a,*], Lu Cai[f,**]

[a] Cancer Center, the First Hospital of Jilin University, Changchun, Jilin 130021, China
[b] Department of Spinal Surgery, the First Hospital of Jilin University, Changchun, Jilin 130021, China
[c] Department of Medical Administration, China-Japan Union Hospital of Jilin University, Changchun, Jilin 130033, China
[d] Heilongjiang Provincial Institute for Food and Drug Control, Harbin, Heilongjiang 150001, China
[e] Department of Clinical Laboratory, the First Hospital of Jilin University, Changchun, Jilin 130021, China
[f] Pediatric Research Institute, and Departments of Pediatrics, Radiation Oncology, Pharmacology and Toxicology, University of Louisville, Louisville, KY 40292, USA

ARTICLE INFO

Keywords:
Resveratrol
Type 1 diabetes
Testis
Apoptosis
Nuclear factor erythroid 2-related factor 2
Kelch-like ECH-associated protein 1
p62

ABSTRACT

Infertility is a common complication in diabetic men, mainly due to the loss of germ cells by apoptotic cell death. However, effective and safe approaches to prevent diabetic induction of testicular apoptosis for diabetic patients have not been available. Resveratrol (RSV), a group of compounds called polyphenols from plants, has been indicated its promising used clinically for cancers and cardiovascular diseases. Therefore, the present study aimed determining whether RSV attenuates type 1 diabetes (T1D)-induced testicular apoptotic cell death in a mouse model. We found that testicular apoptosis and oxidative stress levels were significantly higher in T1D mice than control mice. In addition, the phosphorylation level of metabolism-related Akt and GSK-3β was downregulated and Akt negative regulators PTEN, PTP1B and TRB3 were upregulated in the T1D group. These effects were partially prevented by RSV treatment. Nrf2 and its downstream genes, such as NQO-1, HO-1, SOD, catalase and metallothionein were significantly upregulated by RSV treatment. In addition, RSV-induced Nrf2 activation was found due to Keap1 degradation, mainly reliant on p62 that functions as an adaptor protein during autophagy. These results indicate that the attenuation of T1D-induced testicular oxidative stress and apoptosis by RSV treatment was mainly related to Akt-mediated Nrf2 activation via p62-dependent Keap1 degradation.

1. Introduction

The global increase in the prevalence of diabetes presents significant clinical challenges due to the high rates of diabetic complications and mortality associated with the disease. For instance, according to the National Diabetes Statistics, 30.3 million American individuals, representing 9.4% of the American population, had diabetes in 2015. Furthermore, it is estimated that about 193,000 Americans under the age of 20, representing approximately 0.24% of the American population, have been diagnosed with diabetes. (http://www.diabetes.org/diabetes-basics/statistics/#sthash.XrouwO0y.dpuf). It is known that diabetes is associated with pathological and functional damage to various organs, resulting in a variety of complications. Therefore, the development of efficient approaches to prevent or postpone the development of these complications is critical. Diabetes is significantly associated with infertility in males [1], but effective and safe approaches to prevent diabetic induction of testicular apoptosis for diabetic patients have not been available. Several mechanisms have been proposed for explaining the development of infertility in diabetic men [1–3]; however, germ cell loss may represent the direct and most important contributor to the loss of fertility in diabetic males [4–6].

Testicular apoptotic cell death, which occurs at low levels during normal spermatogenesis, is significantly increased under diabetic conditions [4,7–9]. There is increasing evidence demonstrating that

Abbreviations: RSV, resveratrol; STZ, streptozotocin; ROS, reactive oxygen species; RNS, reactive nitrogen species; SOD, superoxide dismutase; Nrf2, nuclear factor erythroid 2-related factor 2; Keap1, kelch-like ECH-associated protein 1; NQO-1, NAD(P)H:quinone oxidoreductase; HO-1, heme oxygenase 1; CAT, catalase; T1D, type 1 diabetes mellitus; MDA, malondialdehyde; 4-HNE, 4-hydroxynonenal; 3-NT, 3-nitrotyrosine; PTP-1B, protein tyrosine phosphatase-1B; TRB3, Tribbles Homologue 3; MT, metallothionein; qRT-PCR, quantitative real-time polymerase chain reaction; TUNEL, Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling; TdT, terminal deoxynucleotidyl transferase

* Correspondence to: Cancer Center, the First Hospital of Jilin University, 71 Xinmin Street, Changchun, Jilin 130021, China.
** Correspondence to: Department of Pediatrics, the University of Louisville, 570 South Preston Street, Baxter I, Suite 304 F, Louisville, KY 40202, USA.
E-mail addresses: cuijw@jlu.edu.cn (J. Cui), L0cai001@louisville.edu (L. Cai).

https://doi.org/10.1016/j.redox.2017.11.007
Received 4 October 2017; Received in revised form 26 October 2017; Accepted 8 November 2017
Available online 08 November 2017

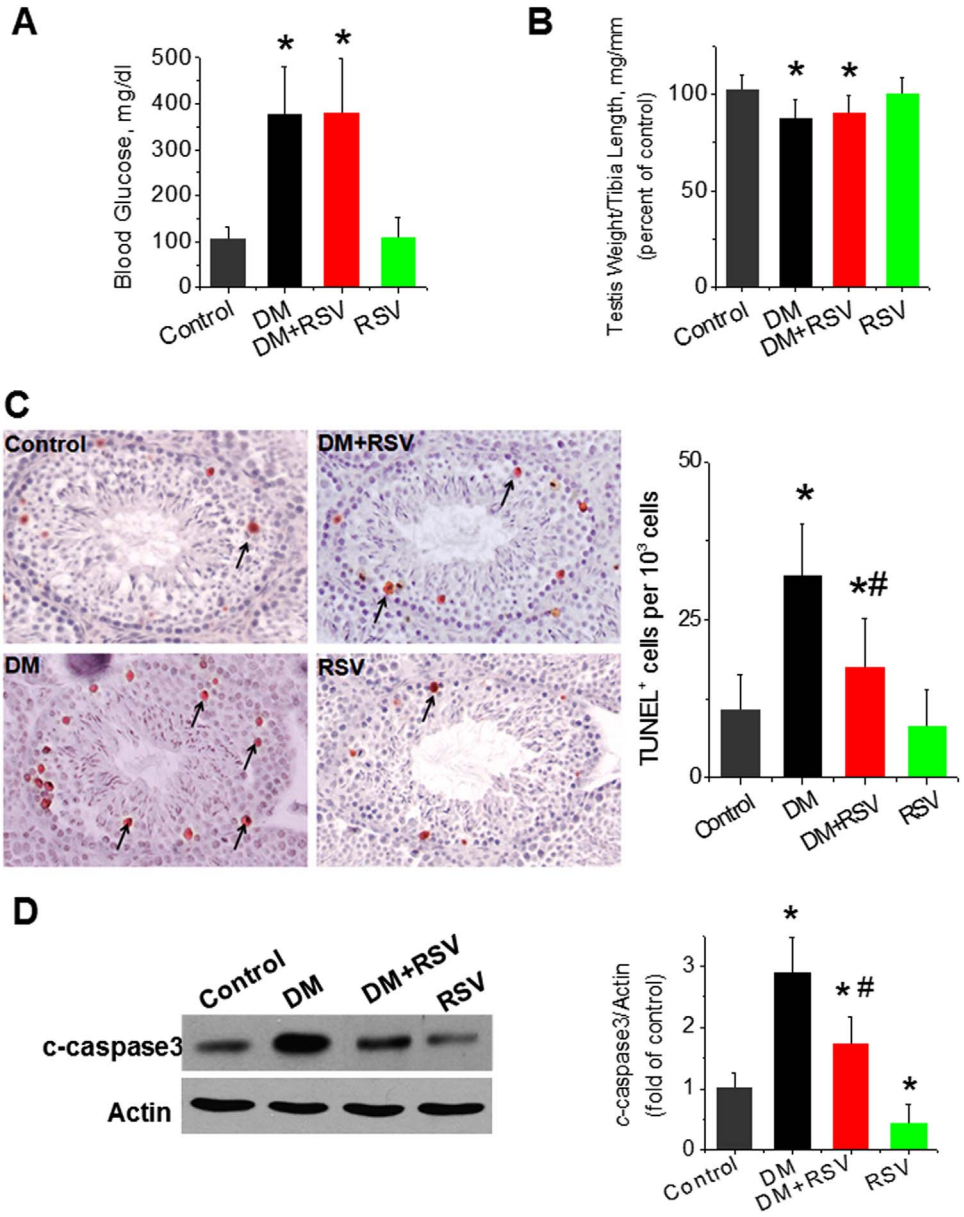

Fig. 1. ***Effects of RSV on T1D-induced testicular apoptosis.*** T1D was induced in mice by multiple low-doses of STZ at 50 mg/kg daily for 5 days. After the onset of hyperglycemia, diabetic and age-matched control mice were treated with or without RSV at 20 mg/kg every other day for 4 months. At the end of the treatment period, blood glucose levels (A), testis weight/tibia length ratios (B) were determined. Testicular apoptotic cell death was examined by TUNEL staining. TUNEL-positive cells were quantitatively analyzed (C). Testicular apoptosis expression was examined by western blotting assay for the expression of cleaved-caspase3 (D). Data are presented as mean ± SD (n = 6 at least in each group). *, $P < 0.05$ vs. control group; #, $P < 0.05$ vs. DM.

testicular apoptotic cell death, which may be induced by the administration of streptozotocin (STZ) in the type 1 diabetic (T1D) rat or mouse model, occurs predominantly via activation of the mitochondrion-mediated cell death pathway [4–6,9–11]. These studies indicate that oxidative stress and damage play a critical role in testicular cell death in diabetic individuals. Oxidative stress occurs in cells or tissues when the excessive generation of reactive oxygen or nitrogen species (ROS or RNS) overwhelms the endogenous antioxidant defense. Therefore, by increasing the antioxidant capacity of the testis tissue would be a potentially efficient approach for preventing and reducing the incidence of testicular apoptotic cell death, and consequently preventing the occurrence of infertility in diabetic males.

Nuclear Factor-Erythroid 2-Related Factor 2 (Nrf2), as a transcription factor, regulates basal and inducible transcription of genes encoding protective molecules against various oxidative stresses [12]. In response to a range of oxidative and electrophilic stimuli including ROS and/or RNS, heavy metals, and certain disease processes, Nrf2 is activated and mediates the induction of a spectrum of cyto-protective proteins including phase II enzymes, such as NAD(P)H: quinone oxidoreductase (NQO-1), catalase (CAT), and superoxide dismutase (SOD), and antioxidant proteins, such as heme oxygenase 1 (HO-1), through the antioxidant response element-dependent pathway. Deletion of the Nrf2 gene was found to cause an age-dependent testicular and epididymal oxidative stress, which disrupts spermatogenesis [13], suggesting a critical role for the transcription factor Nrf2 in preventing oxidative disruption of spermatogenesis. In our previous study, we have also demonstrated a critical role of Nrf2 in preventing diabetes-induced oxidative disruption of spermatogenesis both in T1D and type 2 diabetes (T2D) model [9,14].

The p62 localizes to sites of autophagosome formation and can associate with both the autophagosome-localizing protein LC3 and ubiquitinated proteins [15]. Therefore, p62 is considered to act as a receptor for ubiquitinated proteins, organelles, and microbes, which it sequesters into the autophagosome [16]. The p62 interacts with the Nrf2-binding site of Keap1 and competitively inhibits the Keap1-Nrf2 interaction, which is responsible for the expression of a battery of genes encoding antioxidant proteins and anti-inflammatory enzymes [17,18]. Nrf2 positively regulates p62 gene expression, implying a positive feedback loop [19].

Resveratrol (RSV) is a group of compounds called polyphenols, with