

SIRT-1 ACTIVATOR RESVERATROL PROTECTS HUVECs FROM HIGH GLUCOSE-DEPENDENT REDOX AND GLYCATIVE IMBALANCES





Mijiti M¹, Santini S Jr¹, Cordone V¹, Falone S¹, Amicarelli F¹ ¹ Dept. of Life, Health and Environmental Sciences, University of L'Aquila, Italy



Introduction

The link between diet and health is very interesting, in that it is ascertained that modifications in dietary intake may be important in causing, preventing or delaying the onset of several dysfunctions and diseases. According to national Diabetes Statistics Report, endothelial dysfunctions represent an early-occurring alteration in obesity- and diabetes-related disorders[1].

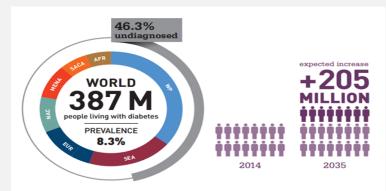




Fig1. Worldwide Diabetic Population estimation [1]

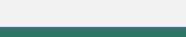
Both redox imbalances and buildup of methylglyoxal (MG), a glycolysis-derived pro-oxidant compound which is a major precursor of advanced glycation end-products (AGEs), are thought to cause the onset of many hyperglycemia-related diabetic complications[3,4]. Stress response to pro-oxidants, DNA repair, and mitochondrial function is crucially regulated by Sir2-related NAD-dependent protein deacylases (sirtuins), among which SIRT1 and SIRT3 play a critical role in cellular and mitochondrial homeostasis[5]. SIRT1 is known to be activated by resveratrol (RSV), a polyphenolic antioxidant compound found in red wine, grapes, and other foods [7]. Several relevant studies reported that RSV evoke prolonged beneficial effects on the endothelial redox balance by modulating the expression of key antioxidant enzymes[8-9]. However, very limited knowledge is available about how RSV may limit or prevent high glucose (HG)-derived imbalances in redox status and anti-glycation defences within human endothelial cells. In particular, no literature data is available as to whether SIRT1 is required by RSV to counteract oxidative and glycative stress in HG-challenged endothelial cells.

Objective



To study the crosstalk between SIRT1 mediated regulation and antioxidative, antiglycative defence systems within endothelial cells.

To study the protective effec of RSV on human umbilical vein endothelial cells (HUVECs) from both redox impairment and



glycative processes that are induced by HG.

Experimental methods

• HUVECs purchased from Lonza (cat. C2519A, pooled donor).

purchased from Sigma-Aldrich (cat. G7021-100G). Glucose

 Resveratrol (RSV) provided by Sigma-Aldrich (cat. R5010-100MG).

Cell amplification

HUVECs were amplified in vitro until passage 3/4.

Cell treatments

Cells were treated with HG and/or RSV.

Biochemical assays

- Protein expression: 1) SIRT1, SIRT3;
- 2) Glyoxalase 1 (GLO1), glyoxalase 2 (GLO2);
- Catalase (CAT), nuclear factor erythroid 2-related factor 2 (NRF2), superoxide dismutase 2 (SOD2).

Results

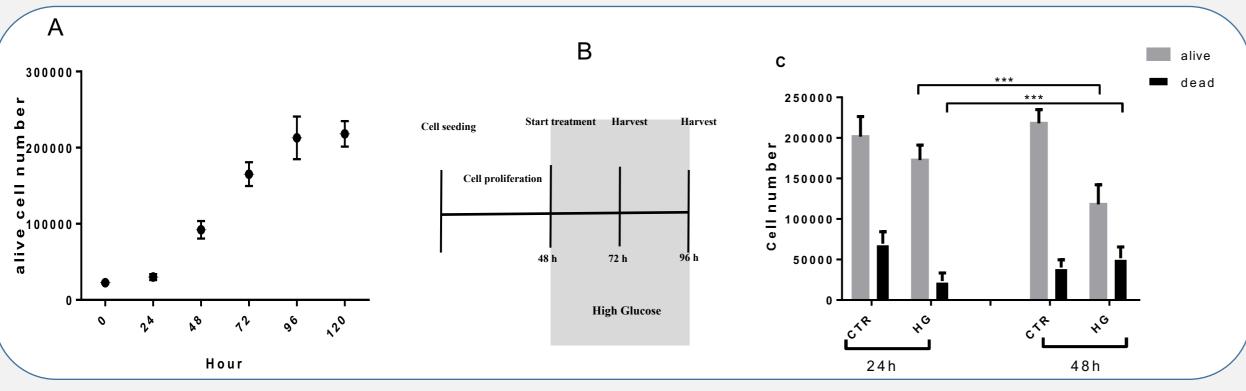


Figure 2. A): HUVECs growth curve. Data was expressed as means ± s.d. (n=4); B) Scheme of the treatment with HG; C) HUVECs treatment with high glucose (25 mM) for either 24 or 48 hours. Two-way ANOVA, ***P<0.01, n=4

The cell growth curve showed us three distinct phases in HUVECs proliferation (i.e., lag phase, exponential phase, and plateau) [Fig. 2A]. On this basis, we decided to start all the treatments after 48 hours from seeding (i.e., during the exponential phase) [Fig. 2B]. Moreover, we found that cells that were incubated with 24-h HG showed less cytotoxicity, as compared to that observed in cells incubated in HG for 48-h [Fig. 2C].

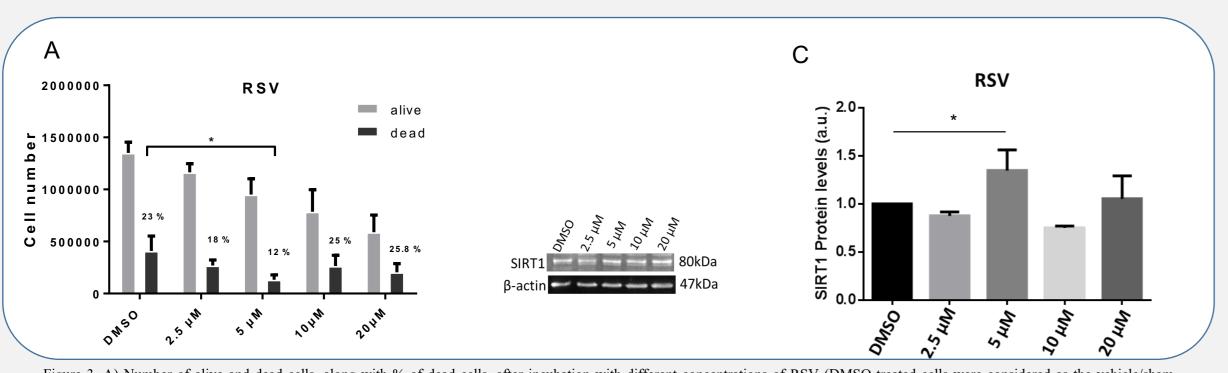
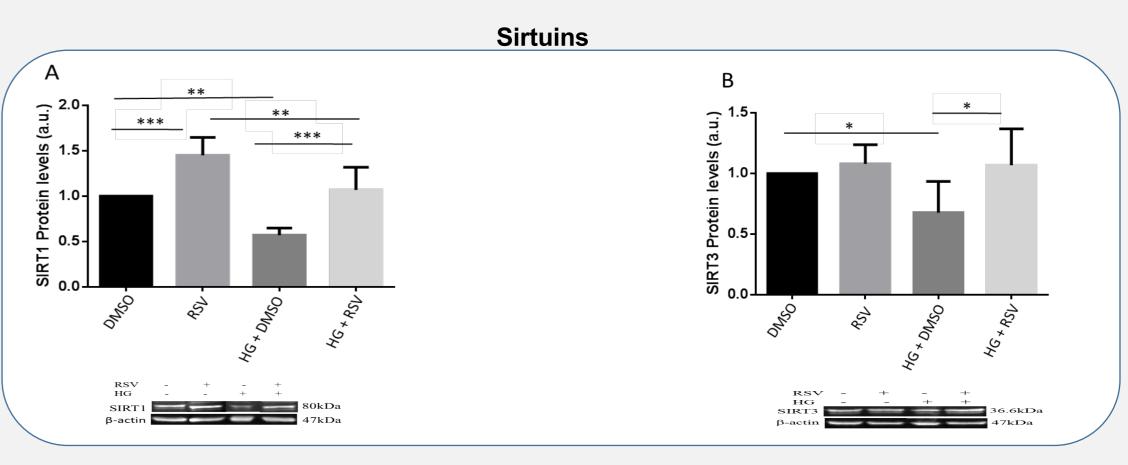


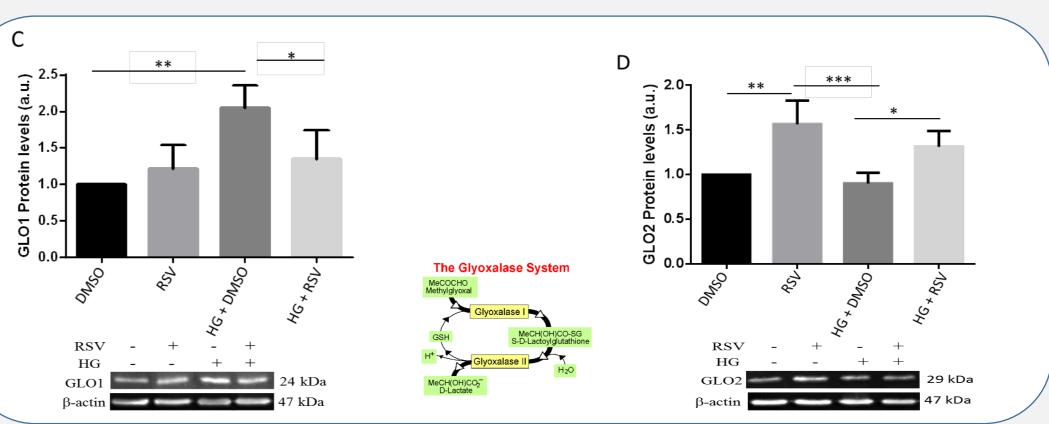
Figure 3. A) Number of alive and dead cells, along with % of dead cells, after incubation with different concentrations of RSV (DMSO-treated cells were considered as the vehicle/shame condition). Data was expressed as means ± s.d. (n=4), One way ANOVA, *P<0.05; B) SIRT1 protein level was assessed by Western immunoblotting, and signals were normalized against βactin. Data was expressed as arbitrary units (means ± s.d.) (n=3, with the exception of 5 µM RSV n=8). One-way ANOVA followed by post-hoc Tukey test for multiple comparisons, *P<0.05.

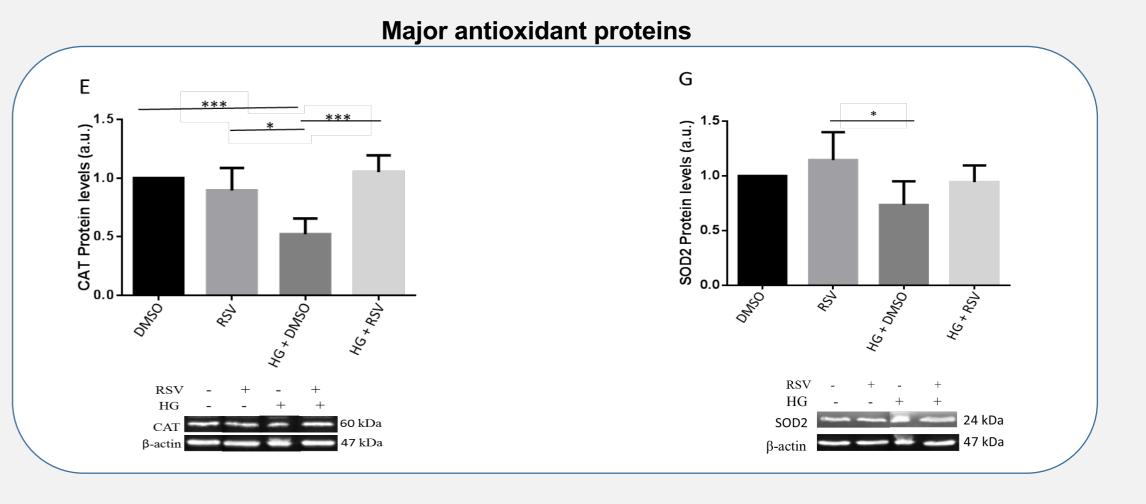
Our results revealed that treatment with 5 µM RSV reduced cell death, as compared to other concentrations [Fig. 3A]. The treatment with 5 µM RSV in normal glucose condition caused the overexpression of SIRT1 protein [Fig. 3B], and this was confirmed by all subsequent experiments.

Results



Glyoxalase system





Redox-responsive transcriptional factor

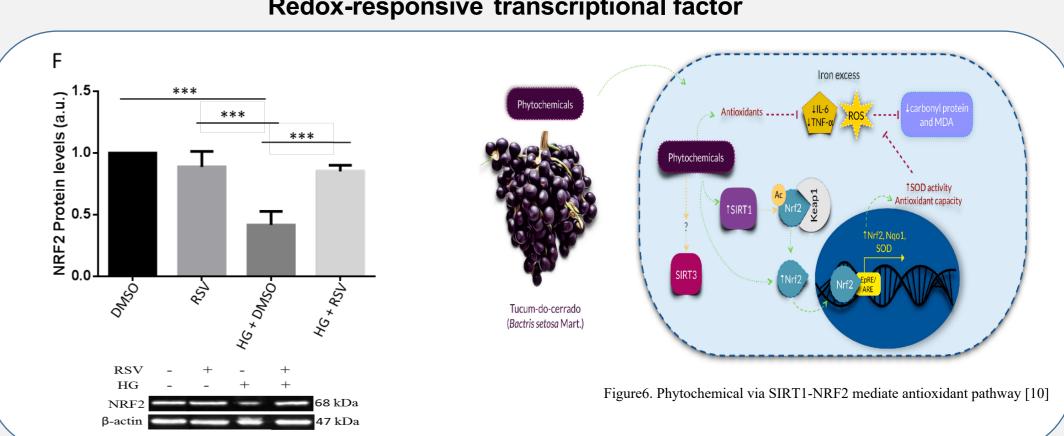


Figure 4. Protein expression of SIRT1 (panel A), SIRT3 (panel B), GLO1 (panel C), GLO2 (panel D), CAT (panel E), NRF2 (panel F), and SOD2 in HUVECs after a 24-h treatment with either 5 µM RSV or vehicle (DMSO), in presence or absence of HG (25 mM). Protein expression was investigated by Western immunoblotting and signals were normalized against β-actin. Data was expressed as arbitrary units (means ± s.d.) (n=4 for all panels, with the exception of panels A and B (n=7 and n=6, respectively)). 2 × 2 factorial ANOVA and post-hoc Tukey test for multiple comparisons, *P<0.05, **P < 0.01, ***P<0.001

The levels of SIRT1, SIRT3, CAT, and NRF2 were significantly down-regulated by HG [Fig. 4A, B, E, and F, respectively]. Interestingly, RSV completely abolished such a negative effect of HG, thus restoring the normal levels of SIRT1, SIRT3, CAT and NRF2. Conversely, GLO1 was found to be greatly increased in cells treated with HG, however this effect was totally reverted by RSV treatment [Fig 4C]. RSV significantly increased GLO2 level in HG condition compared to HG+DMSO [Fig 4D]. Finally, we observed nearly significant reduced SOD2 protein expression after HG treatment, which was reverted by co-incubation with RSV. However, in order to confirm this preliminary results, we are currently replicating SOD2-targeting Western blotting.

Conclusions and Future directions

Overall, our results suggest that the protein expression of the major determinants of antioxidant and antiglycative defence systems in human endothelial cells was impaired by HG. Interestingly, all the HG-induced imbalances were reverted by co-incubation with resveratrol. In particular, we obtained clues that RSV may ameliorate the related pro-oxidant and pro-glycation challenges on HUVECs, possibly by regulating SIRT1–dependent pathway. Nevertheless, further studies are needed to elucidate the underlying mechanisms.



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