SIRT1-dependent response is crucial for the resveratrol-induced upregulation of antioxidant and antiglycative defence in high glucose-challenged HUVECs

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Background

Excessive accumulation of reactive oxygen species (ROS) and unrestrained build-up of glycolysis-derived methlyglyoxal (MG) are thought to be involved in the endothelial dysfunction that precedes diabetes-related vascular complications (1-9). On this basis, researchers are interested in finding strategies to contrast oxidative stress (OS) and glycative stress (GS) by enhancing antioxidative and MG metabolism in high glucose (HG)-challenged endothelium (10-12). Endothelial function in HG is improved by resveratrol (RSV), a natural phytoalexin (13-15), however whether RSV protects HG-challenged endothelial cells mostly via its direct antioxidant effects or by modulating the major antiglycative/antioxidative defence systems remains to be demonstrated. Most importantly, it remains to be proved whether SIRT1, a NAD+-dependent deac(et)ylase critically involved in metabolic adaptation, cell survival and response to cellular stress (16-17), is essential for RSV to protect the endothelial milieu from HG cytotoxicity.

Goals

1) to provide detailed informations about the redox- and, most importantly, the MG-related biomolecular mechanisms possibly underlying the protective effects of RSV in human endothelial cells upon HG;
2) to establish whether SIRT1 is essential for RSV to protect human endothelial cells against HG-dependent cytotoxicity;
3) to demonstrate whether SIRT1 is required for RSV to regulate ROS- and MG-targeting enzymatic systems in human endothelial cells.

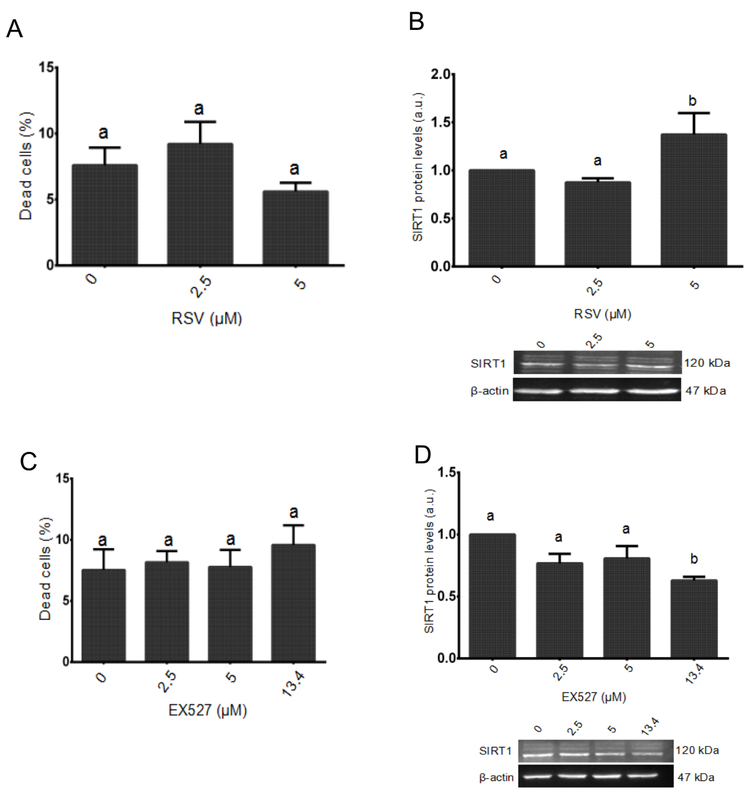
Materials & Methods

Commercially-available human umbilical vein endothelial cells (HUVECs) were kept in 5.55 mM glucose (CTR) or 30.55 mM glucose (HG), and co-incubated with either RSV (5 μM) or RSV+EX527 (SIRT1 inhibitor) (5 μM+13.4 μM), on the basis of concentration-response curves. Cell viability was assessed by Trypan blue staining and phase contrast microscopy. Apoptosis was assessed via Annexin V/PI double staining and IncuCyte-based microscopy imaging. Morphological assessment was performed by scanning electron microscopy (SEM). Expression and function of SIRT1, SOD1, SOD2, CAT, and GLO1 were studied by quantitative relative real time RT-PCR, Western blotting (WB), and spectrophotometric enzymatic assays. Oxidative damage was evaluated by measuring TBARS, and the MG-dependent protein damage was evaluated by anti-argpyrimidine-based WB.

Results

Fig.1

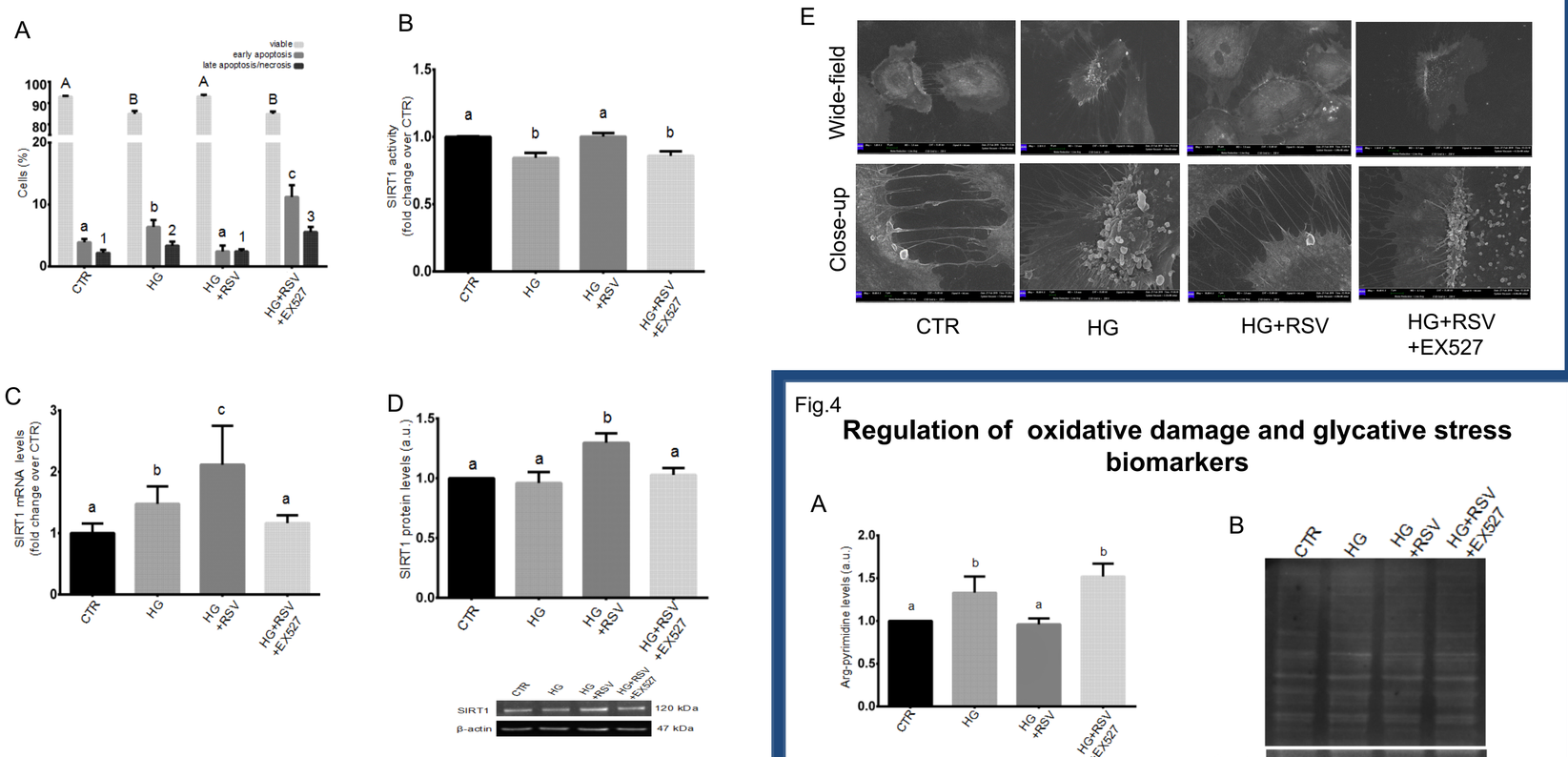
RSV increases / EX527 decreases SIRT1 expression with no cytotoxic effects



Same letters indicate no statistically significant difference (One-Way ANOVA); a vs b, P<0.05.

Fig.2

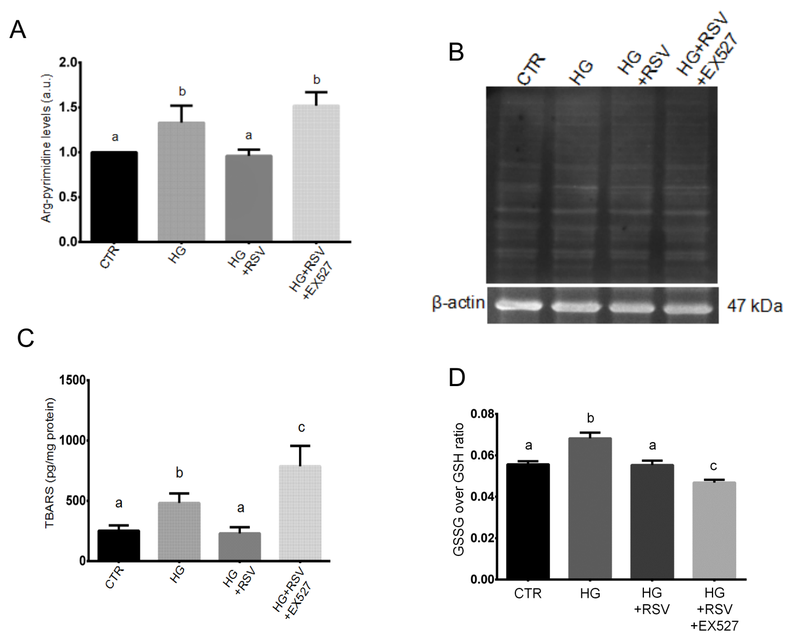
SIRT1 activation is essential for RSV to elicit cytoprotective effects upon HG



Same letters indicate no statistically significant difference (One-Way ANOVA); a vs b, P<0.01; b vs c, P<0.001; a vs c, P<0.01.

Fig.4

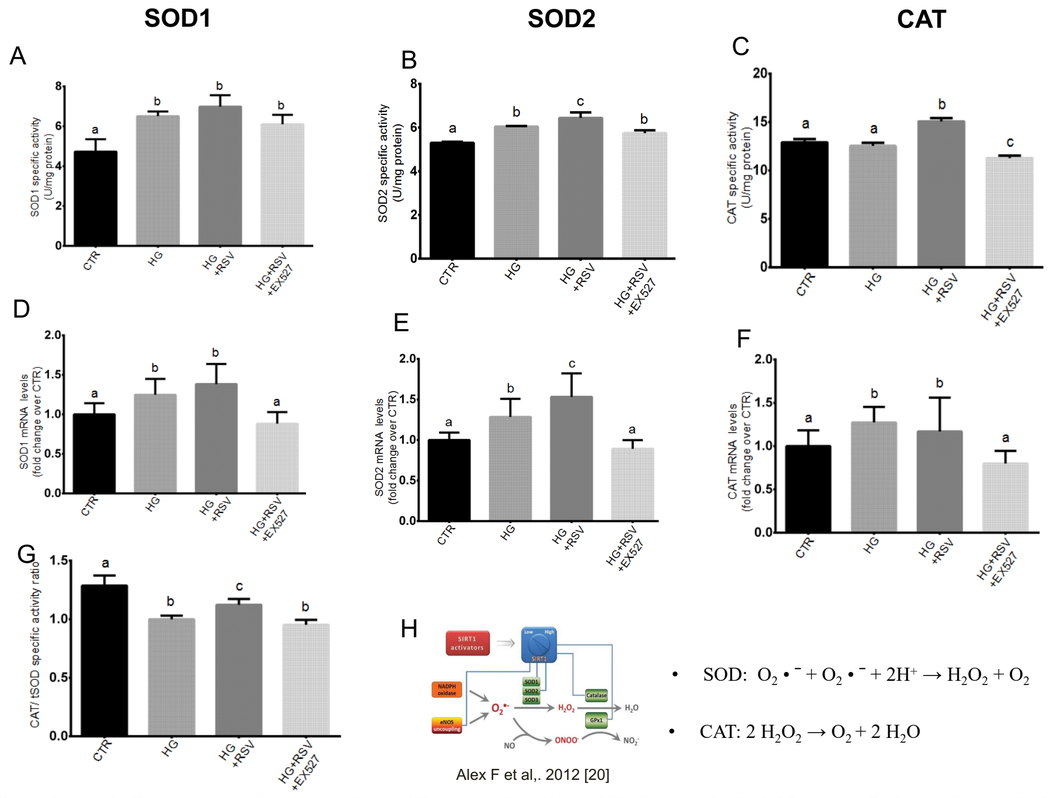
Regulation of oxidative damage and glycative stress biomarkers



Same letters indicate no statistically significant difference (One-Way ANOVA); a vs b, P<0.05; a vs c, P<0.001; b vs c, P<0.01.

Fig.4

SIRT1-mediated impact of RSV on antioxidant enzymes



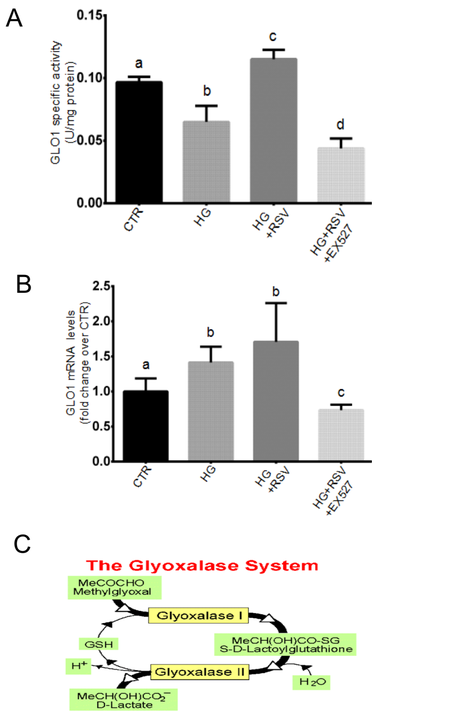
- SOD: $O_2 \bullet^- + O_2 \bullet^- + 2H^+ \rightarrow H_2O_2 + O_2$
- CAT: $2\,H_2O_2 \rightarrow O_2 + 2\,H_2O$

Alex F et al,. 2012 [20]

Same letters indicate no statistically significant difference (One-Way ANOVA); a vs b, P<0.05; a vs c, P<0.001; b vs c, P<0.01.

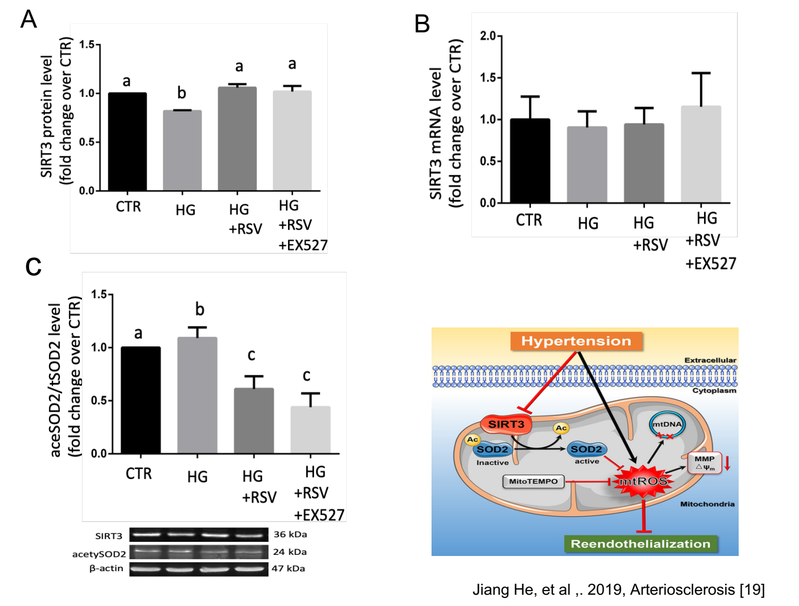
Fig.5

SIRT1-mediated impact of RSV on glyoxalase system



Thornalley et al , . 2014, diabetes [18]

Effects of RSV on SIRT1-SIRT3-mtSOD axis



Jiang He, et al ,. 2019, Arteriosclerosis [19]

Same letters indicate no statistically significant difference (One-Way ANOVA); a vs b, P<0.05; a vs c, P<0.001; b vs c, P<0.01.

Conclusions

- HG-challenged HUVECs show increased oxidative/glycative damage, and this is paralleled by impaired scavenging of ROS and MG;
- RSV rescues the HG-induced impairment of ROS and MG scavenging, as well as prevents the pro-oxidant and pro-glycation effects triggered by HG;
- The up-regulation of SIRT1 is essential for RSV to protect HUVECs from HG cytotoxicity, and to elicit antioxidant/antiglycative effects on HG-challenged HUVECs;
- The SIRT1-SIRT3-mtSOD axis may be suggested as a new pathway to enhance mitochondrial biogenesis in the mechanisms of action of RSV.

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