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 and glycative stress by enhancing ROS 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 it still has to be established whether RSV protects HG-challenged endothelial cells mostly via its direct antioxidant effects or by modulating the major antiglycative/antioxidative defence systems. Most importantly, it remains to be clarified whether SIRT1, a NAD+-dependent deac(et)ylase critically involved in metabolic adaptation, cell survival and response to cellular stress (16-22), is essential for RSV to protect the endothelial milieu from HG cytotoxicity.

Goals

1) to provide detailed informations about redox- and, most importantly, MG-related biomolecular mechanisms through which protective effects of RSV in HG-challenged endothelial cells are elicited;
2) to establish whether SIRT1 is essential for RSV to protect 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.

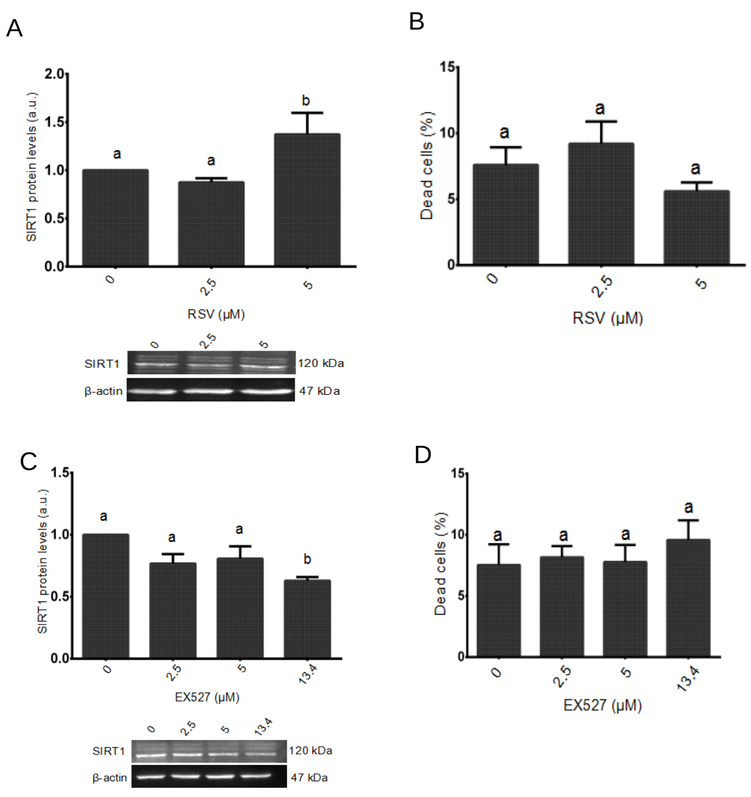
Materials & Methods

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) for 24 h, on the basis of concentration-response curves. Cell viability was assessed by Trypan blue staining (23). Apoptosis was assessed via Annexin V/PI double staining (24) 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 (25,26), Western blotting (WB) (27,28), and spectrophotometric enzymatic assays (29-33). Reduced and oxidized glutathione levels were measured according to a photometric method (34). Oxidative damage was evaluated by measuring TBARS (35), and the MG-dependent protein damage was evaluated by anti-argpyrimidine-based WB (36).

Results

Fig.1

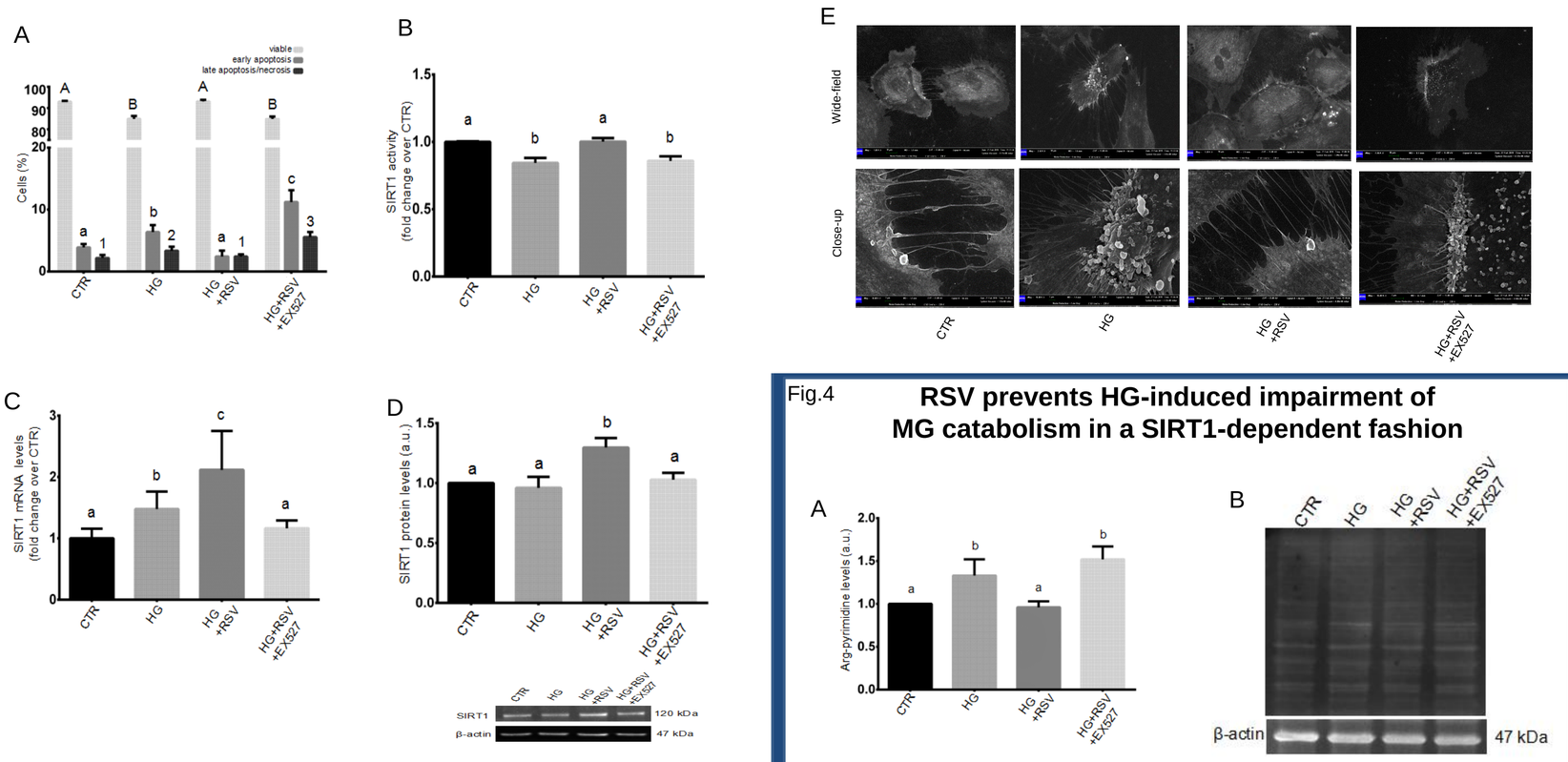
RSV increases / EX527 decreases SIRT1 expression with no cytotoxic effects



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

Fig.2

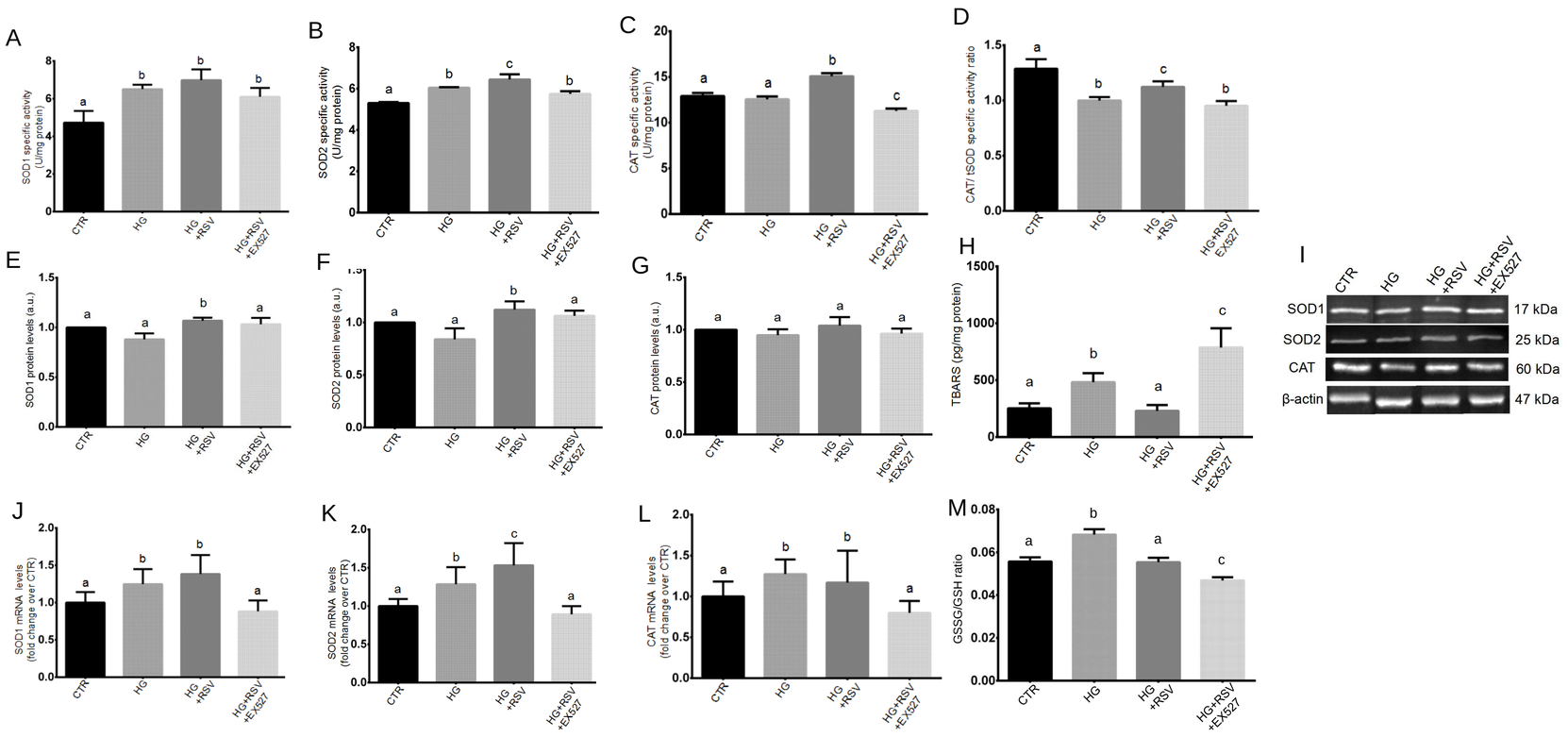
SIRT1 activation is essential for RSV to elicit cytoprotective effects upon HG



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.3

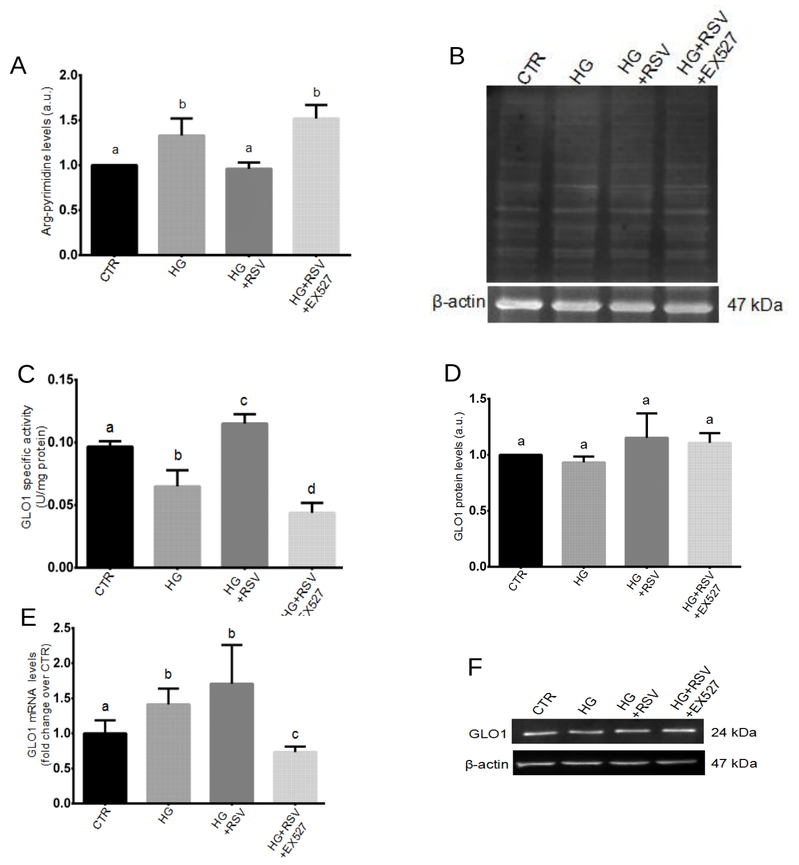
RSV reverts HG-induced impairment of ROS-targeting enzymes in a SIRT1-dependent fashion



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 (A-L), a vs b, P<0.001; a vs c, P<0.001; b vs c, P<0.001 (M).

Fig.4

RSV prevents HG-induced impairment of MG catabolism in a SIRT1-dependent fashion



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 redox imbalance, as well as increased oxidative/glycative damage, and this is associated with impaired scavenging of ROS and MG;
- RSV rescues the HG-induced impairment of ROS and MG scavenging, as well as prevents the redox imbalance and the pro-oxidant/pro-glycation effects elicited by HG;
- SIRT1 up-regulation is essential for RSV to protect HUVECs from HG cytotoxicity, and to trigger antioxidant/antiglycative response in HG-challenged HUVECs.

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