Prolactin protects beta cells against oxidative stress through HSPB1

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Background and aims: Maintaining islet cell viability in vitro, although challenging, appears to be a strategy for increasing the outcome of pancreatic islet transplantation. We have shown that heat shock protein B1 (HSPB1) mediates prolactin (PRL) beta-cell inhibition of apoptosis. Since the role of HSPB1 in beta-cells is still unclear, we explored the molecular mechanisms by which HSPB1 mediates PRL-induced beta-cell cytoprotection.
Materials and methods: Wild type, HSPB1 silenced or overexpressing MIN6 cells were used as beta-cell models. Biochemical and cell biology parameters such as protein levels, oxidative stress quantification and cell viability were analysed by HPLC-mass spectrometry, western blotting, and fluorescent bioassays among other techniques.
Results: Lysates from PRL and/or cytokine-treated MIN6 beta-cells were subjected to HSPB1 immunoprecipitation. Of the 130 client proteins identified by mass spectrometry, 60 were interacting with HSPB1 under both situations whereas 49 were only detected in the presence of PRL and cytokines. Of note were oxidative stress resistance proteins such as MnSOD, CuZnSOD and PRDXs. We then investigate whether HSPB1-knocked down cells would show a different sensibility towards oxidative stress. Our results indicated not only that PRL was able to protect both control MIN6 cell lines against menadione-induced toxicity (EC50: 11.88 and 14.97 μM for control and PRL treated cells respectively), but also that this effect was mediated by HSPB1, since its silencing completely abrogated PRL's effect on cytoprotection (EC50: 11.61 and 11.91 μM for control and PRL, respectively). Using cells expressing cytosolic or mitochondrial variants of the D-amino acid oxidase (DAAO), we observed that HSPB1 was important mainly for the protection against ROS produced in mitochondria displaying an even greater (around 40%) decrease in the EC50 of MIN6-shHSPB1 cells when compared to that of control cells. HSPB1 silenced cells presented a higher mitochondria-targeted hydroethidine mean fluorescence signal than control cells (at 14 μM menadione. MIN6: 363.7%: MIN6-Sc: 347.7%; MIN6-shHSPB1 cells: 705.4%). HSPB1 overexpression led to opposite effects such as a significant increase of the EC50 of both menadione (16.0μM vs. 14.49 μM for control cells) and H2O2 (32.58 μM vs. 26.2 μM for control cells) and also a reduced overall oxidative stress shown by DCF fluorescence. PRL treatment, HSPB1 silencing or overexpression did not change the expression of antioxidant enzymes; but influenced glutathione cell content reduction state (GSH/GSSG ratio was decreased in shHSPB1 cells by 50% compared to control cells) and glucose-6-phosphate dehydrogenase (G6PD) activity (MIN6-shHSPB1 cells displayed approximately 40 % lower levels of G6PD activity).
Conclusion: We have shown that HSPB1 is important for pro-survival effects against ROS-induced beta-cell death. Altogether our results outline the importance of further studies investigating the importance of HSPB1 for beta-cell viability, since this could lead to the mitigation of beta-cell death through the up-regulation of an endogenous protective pathway.
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