# Prolactin-induced Pro-survival Effects on Pancreatic Beta-Cells are Mediated by HSP25/27.

Wailemann R.A.M. [1]; Terra L.F. [1]; Oliveira T.C. [1]; Santos A.F. [1]; Sogayar M.C. [1,2]; Labriola L.[1]

[1] Biochemistry Department, Chemistry Institute University of São Paulo, São Paulo, Brazil

[2] Cell and Molecular Therapy Center (NUCEL/NETCEM), Medicine School, University of São Paulo, São Paulo, Brazil

**Introduction:** We have previously shown that recombinant human prolactin (rhPRL) inhibits beta-cell apoptosis. Moreover, we have recently reported PRL-induced up-regulation of the anti-apoptotic HSP25/27protein in human islets. Since the function of HSP25/27 in beta-cells has not been directly studied, we set out to explore the role of HSP25/27 in prolactin-induced beta-cell cytoprotection. **Material and Methods:** We used parental and HSP25 knocked-down Min6 cells, which were subjected different treatments and analysed by flow cytometry and assessed for protein levels and caspase activity. **Results and Discussion:** Our data showed that upon treatment with both cytokines and rhPRL, the proportion of fragmented nuclei was increased in HSP25 silenced cells when compared to control cells. The inhibition of cytokine-induced caspase-3 activity as well as of caspase-9 and Bax protein levels mediated by rhPRL in wild type cells was reverted in knocked-down cells. The kinetics of HSP 25/27 and HSTF1 expression levels were studied in primary cultures of human pancreatic islets which were serum starved and treated with rhPRL for short periods of time, showing that while HSTF1 presented an increase in protein expression level after 10 min of rhPRL treatment, HSP27 reached its maximum expression level upon 2h of hormonal treatment. An increase in STAT1 phosphorylation level was detected after 10min of rhPRL treatment reaching the highest levels upon 30 min of treatment. **Conclusion**: We demonstrated a key role for HSP25/27 in rhPRL-induced cytoprotective effects, since the lack of this protein abolished the beneficial effects induced by PRL in beta-cells. We provided, for the first time, evidence for co-regulation of HSP27 and HSTF1 upon rhPRL treatment of human pancreatic beta-cells, an effect which could be mediated by activated STAT1. Our results could lead to the mitigation of beta-cell death through the up-regulation of an endogenous protective pathway which is not dependent on the modulation of the immune system.

Key words: islet cell biology, islet transplantation, recombinant human prolactin, (HSP)25/27 heat shock protein, beta-cell cytoprotection.

Support: BNDES, CNPq, FAPESP, FINEP,PRP-USP , MCTI, MS-DECIT.

Abbreviations: (HSP) Heat Shock Protein; (HSTF1) Heat Shock Transcription Factor 1; (STAT1) Signal Transducer and Activator of Transcription 1; (rhPRL) recombinant human prolactin.