Molecular mechanisms involved in cytoprotection, autophagy and malignant transformation of human beta-cells.

Letícia F. Terra [1], Miguel H. Garay-Malpartida [2], Rosangela A. M. Wailemann [1], Priscila C. Teixeira [3], Aline P. Bordino [1], Ancély F. Santos [1], Talita C. Oliveira [1], André Zelanis [4], Edecio Cunha-Neto [3], Jorge Kalil [3], Martin R. Larsen [5], Giuseppe Palmisano [5,6], Mari C. Sogayar [1] and Leticia Labriola [1]

[1]Instituto de Química, Departamento de Bioquímica – Universidade de São Paulo (USP), São Paulo, Brasil; [2]Escola de Artes, Ciências e Humanidades (EACH) - USP, São Paulo, Brasil; [3]Instituto do Coração, Faculdade de Medicina – USP, São Paulo, Brasil; [4]Laboratório Especial de Toxinologia Aplicada (CAT/CEPID) - Instituto Butantan, São Paulo, Brasil; [5]Biochemistry and Molecular Biology Department – University of Southern Denmark, Odense, Denmark; [6]Instituto de Ciências Biomédicas – USP, São Paulo, Brasil

Transplantation of pancreatic islets constitutes an alternative for type 1 diabetes. However, it is limited by the shortage of organ donors. We investigated the role of recombinant human prolactin (rhPRL), shown to have beneficial effects on beta-cells, in their survival. Human pancreatic islets were isolated, pre-treated in the absence or presence of rhPRL and subjected to serum starvation or cytokine treatment. Apoptotic beta-cells, evaluated using flow cytometry, quantitative RT-PCR, western blot and fluorimetric assays. were decreased in the presence of rhPRL. Cytoprotection involved an increase of BCL2/BAX ratio, and inhibition of caspase-8, -9 and -3. Our study provides new direct evidence for a protective effect of lactogens on human beta-cell apoptosis. These findings are relevant for improvement of the islet isolation procedure and for clinical islet transplantation. Taking into account the known relationship between cytokines and DM1 and recent observations suggesting a role for autophagy in the development and prevention of DM1, we investigated the role of rhPRL treatment on autophagy markers in INS-1E cell cultures and noticed that rhPRL increased the levels of p-mTOR and decreased autophagosome formation after exposure to both cytokines and ER stressors in beta-cells. Considering the demand for human cells for deeper beta-cells studies, we generated cell lines derived from human insulinomas which secrete hormones and express markers similar to their original tissue. Moreover, we set out to further characterize these lineages by comparing them to primary beta-cells using two-dimensional gel electrophoresis coupled to mass spectrometry. Less than 1% of proteins exhibted differential expression. Proteins Almost all proteins upregulated in insulinoma cells, as MAGE-A2, were first described here and could be related to cell survival and resistance to chemotherapy. In conclusion, our results may provide knowledge to prompt research towards the establishment of bioengineered human beta-cells, and the development of new therapeutic strategies for insulinomas.

Keywords: diabetes, beta-cells, insulinoma