Tackling the challenges posed by type 1 Diabetes mellitus: several ways to skin a cat.

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Type 1 Diabetes mellitus (T1D) arises from autoimmune destruction of pancreatic insulin-producing β-cells, causing severe hyperglycemia, which may lead to vascular diseases, kidney failure, blindness and amputations. Insulin therapy is generally effective requiring strict patient adherence, however, in patients with brittle diabetes, only whole pancreas and islet cell transplantation allow circumventing the poor metabolic control, requiring lifelong patient immunosuppressive treatment, which often causes serious side effects. Islet encapsulation is an attractive alternative to avoid immunosuppression, since the biomaterials used offer a physico-chemical semi-permeable barrier against the attack from immune and inflammatory cells and a source of bioactive molecules. Using a model of Balb/c mice rendered diabetic by streptozotocin injection, which specifically destroys insulin-producing pancreatic β-cells, we showed that: rat islets encapsulated in Biodritin (biopolymer of alginate and chondroitin sulfate) revert diabetes in the absence of immunosuppression; rat islets encapsulated in a new biopolymer (Bioprotect®, patent pending), revert diabetes more efficiently; a novel biomaterial, which we named BioSafe® (patent pending), displayed an even better performance in vitro, and is currently being tested in vivo. However, since human pancreata are scarce, new sources of insulin-producing cells are in great demand. We chose murine totipotent embryonic stem cells (mESCs) to attempt to generate insulin-producing cells (IPCs) in vitro. Employing a published protocol, we generated clusters of IPCs, but were unable to revert diabetes in mice, however, after encapsulation in Bioprotect®, in vivo incubation and retrieval, these encapsulated clusters behaved more like fully matured β-cells in oral gluco tolerance tests, indicating this to be a valid approach for T1D reversion. Large-scale Transcriptomic analysis of mESCs versus IPCs, using commercially available microarrays, revealed 600 differentially expressed upregulated genes and similar amounts of downregulated genes during differentiation. Bioinformatic analysis and ranking led us to elect one of each class: PCP4 (upregulated) and Txinp (downregulated) for functional analysis and to list the 30 most highly expressed genes in IPCs for miRNA analysis. PCP4 is 1,000 times more differentiated in differentiated IPC clusters than in undifferentiated mESCs. Overexpression of PCP4 in mESCs subjected to differentiation into IPCs increased the expression of genes related to β-cell differentiation, such as the Is/l gene (p<0.01), which is important for islet-like aggregates formation. Lentiviral vectors were used for inhibiting Txinp gene expression to establish genetically modified insulinoma cell lines containing shRNA sequences for inhibition of the Txinp gene. TXNIP acts as an endogenous negative regulator of an antioxidant system that protects cells from redox stress. Since pancreatic β-cells are known for their poor antioxidant defenses, we hope to improve mESCs to IPCs differentiation by modulating TXNIP. Among those 30 highly expressed genes in IPCs, an extensive micro RNA analysis was undertaken along the process of differentiation using bioinformatic tools and the Metacore™ platform (Thomson Reuters) to generate a list of predicted miRs, paired with their putative mRNAs, aiming to identify important regulatory pathways involved in IPCs differentiation. Bioengineering and Regenerative Medicine have recently been proposed as potential alternative therapeutic approaches for T1DM. Human pancreas may be decellularized, the remaining bioscaffold, a skeleton of extracellular matrix (ECM), providing an optimal microenvironment for islets formation from isolated and cultured β-cells or from stem cells differentiated into IPCs, aiming at transplantation. We have been decellularizing both human and rat pancreas using detergent solutions to produce bioactive matrices, which will be fully characterized in vitro before reconstitution with either pancreatic cell lines or human acinar and primary β-cells or murine IPC aggregates and implantation into diabetic animals. Financial Support: BNDES, CAPES, CNPq, FAPESP, FINEP, MCTI, MS-DECIT

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