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Autore	SULEIMAN, MARA
Titolo	Human beta cell dysfunction induced by different lipoglucotoxic conditions may be transient or persistent and associates with specific transcriptomic changes which are shared in type 2 diabetes [Tesi di dottorato]
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Sommario	Pancreatic beta-cell failure is determined by the interplay of genetic and acquired factors and represent the key event that leads to the development and progression of type 2 diabetes (T2D). The cause of this derangement is not completely clarified. Work in vivo shows evidence that alleviation of metabolic stress, through low calorie diet, administration of glucose-lowering drugs or bariatric surgery, can improve beta-cell function. Studies in vitro demonstrated that prolonged exposure to saturated fatty acids (lipotoxicity), high glucose (glucotoxicity) or combinations thereof (lipoglucotoxicity) may contribute to beta-cell failure, possibly via endoplasmic reticulum (ER) stress, oxidative stress, loss of identity and/or other mechanisms. In addition, the molecular mechanisms underlying the persistence or transience of human beta-cell defects are still to be investigated to unveil to which extent the functional and molecular modifications (and possible recovery) in non-diabetic (ND) islets reflect those of islets from T2D individuals. The present thesis aimed first to assess the direct impact of different lipoglucotoxic treatments on human beta-cell function, and then to evaluate if the deleterious effects were persistent or reversible after washout. Finally, the associated transcriptomic changes were analyzed and compared

with T2D islet gene expression signature. Islets obtained from 26 ND organ donors were cultured in M199 medium, containing 5.5 mmol/l glucose, for 2 days (D2); then batches of islets were cultured for 2 additional days (D4) either in absence (ctrl) or in the presence of: 0.5 palmitate (P), 11.1 mmol/l glucose (g), 22.2 mmol/l glucose (G), 0.5 mmol/l palmitate + 11.1 mmol/l glucose (P+g), 0.5 mmol/l palmitate + 22.2 mmol/l glucose (P+G), 1.0 mmol/l palmitate + oleate, (1:2 molar ratio, P+O), 1.0 mmol/l palmitate + oleate + 11.1 mmol/l glucose (P+O+g), and 1.0 mmol/l palmitate + oleate + 22.2 mmol/l glucose (P+O+G). At D4, islets were washed and incubated with plain M199 medium for 4 additional days (D8 control and D8 washout, according to the incubation condition). Furthermore, 28 ND and 58 T2D islet preparations were studied. Glucose-stimulated insulin secretion (GSIS) from all the preparations was assessed, and islets were also prepared for RNA extraction and, in selected cases, for histology. No significant change occurred in GSIS with ctrl, g, P+O and P+O+g islets throughout the study period. However, GSIS at D4 declined ($p<0.05$ or less) with P, G, P+g, P+G and P+O+G exposure. Normalization of GSIS was observed at D8 washout vs D4 with P, G and P+g, but not with P+G and P+O+G. For P, G and P+G conditions, islet transcriptome and genome features were analyzed by RNA-sequencing and eQTL, respectively, to unveil the molecular mechanisms underlying beta-cell damage and its reversal. For the conditions where islets were exposed to P (functionally impaired at D4 and rescued at D8 washout), treated islets compared to control islets at D4 had 646 differentially (FDR<0.05) expressed genes (272 up- and 374 downregulated). Of these, 595 were protein-coding genes (248 up- and 347 downregulated by palmitate), including genes involved in lipid metabolism, inflammation and other cell functions. Enrichment analysis identified several P-modified functional categories including upregulation of unfolded protein response, acyl-CoA biosynthesis and fatty acid metabolism and others. Comparison of D8 washout vs D4 palmitate-treated islets identified 714 genes differentially expressed (167 up- and 547 down-). Of these, 656 were protein-coding (142 up- and 514 down-) comprising genes with a role in lipid/glucose metabolism, transcription, inflammation, beta-cell function and others. The Enrichment Map regarding D8 washed out islets showed downregulation of fatty acid metabolism and upregulation of carbohydrate catabolic processes. As for human islets exposed to G (impaired GSIS at D4 with recovery after washout), the comparison of G-exposed islets vs control islets at D4 showed 50 differentially expressed genes (38 up- and 12 down-); among these, 42 were protein-coding (32 up- and 10 downregulated by high glucose) including genes involved in metabolic pathways, gated channel activity and other cell functions. Enrichment analysis showed inhibition of cell junction and metabolic processes. D8 washout vs D4 G-treated islets RNA-seq data comparison resulted in 341 differentially expressed genes (81 up- and 260 down-regulated). Of these, 320 were protein-coding (70 up- and 250 down-), also involved in extracellular organization, establishment of protein localization to ER and glycerophospholipid metabolism. The relative Enrichment Map showed downregulation of response to wounding, myeloid cell differentiation and chemotaxis. More profound changes in islet transcriptome were observed with combined P+G (beta-cell dysfunction at D4 and persistence after washout). D4 P+G-exposed islets resulted in differential expression of 1,498 genes (756 up- and 742 down-). Of these, 1,386 were protein-coding (699 up- and 687

	down-) and they were mainly related to transcription, inflammation, cell turnover, ion channels/transporters, mitochondrial function, and redox balance. The Enrichment Map showed clustering of interrelated gene-sets upregulated for most and comprising the unfolded protein response, protein degradation, mRNA splicing regulation and ER stress-induced apoptosis. The RNA-seq data of human islets at P+G D8 washout vs D4 treated identified only 322 genes differentially expressed (120 up- and 202 down-). Of them, 292 were protein-coding (102 up- and 190 down-). Finally, the molecular changes associated with persistent or transient beta-cell insulin secretion defects were correlated with those of human islets from T2D donors compared with ND donors. The Rank-Rank Hypergeometric Overlap (RRHO) approach was used, which allows to compare differentially expressed transcriptomes between independent experiments. Overall, the RRHO analyses showed that persistent or transient human beta-cell dysfunction induced by metabolic stress was accompanied by specific gene expression signatures that were shared with T2D, with the greatest concordant overlap between conditions that induce beta-cell dysfunction and fail to recover after washout, namely P+G and P+O+G. These results, obtained during the PhD course, show that certain lipoglucotoxic conditions may induce persistent or reversible beta-cell dysfunction, depending on the type, concentration and combination of the stressors. This associates with specific molecular changes that overlap with T2D islet traits. Identification of novel mechanisms responsible for human beta-cell functional deterioration and rescue, which are shared in T2D, could provide novel insights into T2D pathogenesis and should foster the development of improved beta-cell specific therapeutic approaches.
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