## Abstract

Diabetes mellitus is a group of metabolic conditions characterized by elevated blood glucose levels resulting from problems with insulin secretion, insulin action, or both. Morbidity and mortality for this disease has reached values which were considered inconceivable 25 years ago and the consequence involving cardiovascular disease, neuropathy, nephropathy, retinopathy, Alzheimer's disease and others, has dramatic effects on health-quality and life-span in humans. Currently, there are ~28 million patients at age 20 and older affected by diabetes in the United States alone with an incidence of 180,000 new cases per year. Moreover, 186,000 individuals, younger than 20 years of age, have diabetes, and each year ~15,000 are diagnosed with type-1 diabetes. Additionally, more and more children have type-2 diabetes, a condition commonly identified in adults 40 years of age and older. The International Diabetes Federation reports that there are 537 million persons aged 20-79 globally who have diabetes. The number is projected to increase to 643 million by 2030 and 783 million by 2045.

Type-1 diabetes is defined as a disease of progressive autoimmune destruction of β-cells, resulting in severe insulin deficiency. However, long-term type-1 diabetes does not lead to a complete loss of β-cells; β-cells have been found at autopsy, years after the onset of diabetes, and insulin is formed in the majority of diabetic patients following a mixed-meal tolerance test. The synthesis and secretion of endogenous insulin has protective effects on complications and hypoglycemia, indicating that, albeit at low level, functional β-cells are preserved with type-1 diabetes. These β-cells are either protected by the immune reaction or undergo continuous regeneration. The latter possibility is consistent with the expression of cell cycle proteins in β-cells of autopsy samples collected from patients who had type-1 diabetes for more than 50 years. However, whether replicating β-cells are the progeny of stem cells or derive from division of mature β-cells is unknown and an essential question. The loss of β-cells determines the pathology of diabetes and reconstitution of the β-cell compartment alleviates diabetes and its consequences. There is a strong correlation between the residual β-cell mass and the gravity of diabetes and its complications. Two opposite opinions dominate the biology of β-cells: a) β-cells are not terminally differentiated post-mitotic cells, but rather a cell pool that, despite its highly sophisticated function, can reenter the cell cycle and divide; and b) β-cells are irreversibly withdrawn from the cell cycle and can only be formed by activation and lineage specification of stem cells.

Diabetes is characterized by multiple factors involving physiological, biochemical, chemical mediators operating concurrently with, triggered by the same or different pathways. Small molecules kinase inhibitors opened new opportunities for functional studies and regenerative therapies. DYRK1A is one of five members of the Dual-specificity tyrosine (Y) phosphorylation-Regulated Kinase (DYRK) family. The *dyrk1a* gene is located in the Down syndrome critical region and regulates cellular processes related to proliferation and differentiation of neuronal progenitor cells during early development. This has primary focused the research on to its role in neuronal degenerative diseases, including Alzheimer's and Down syndrome. However, recent studies have shown a role of DYRK1A in diabetes. Research conducted with mice that have haploinsufficiency in the *dyrk1a* gene has provided evidence supporting the notion that these animals exhibit significant glucose intolerance, a decrease in the mass and proliferation of β-cells, ultimately resulting in the development of diabetes. The effect was seen to be greatly enhanced by the upregulation of DYRK1A in β-cells

This dissertation follows current research trends that are mainly concerned with the characterization of novel small-molecule DYRK1A kinase inhibitors and the application of pancreatic β -cell organoids in diabetes studies. The main goals of the work are: (1). To identify and characterize the structural properties of small-molecule inhibitors of the DYRK1A kinase. (2) To evaluate the efficacy of these inhibitors in inhibiting DYRK1A *in vitro*. (3) To analyze the potential therapeutic effects of these inhibitors in pancreatic β-cell organoids models for diabetes. (4) To assess the effects of selected inhibitors on insulin secretion in an organoid model of human pancreatic islets. (5) To compare the activity of the tested inhibitors with harmine, currently the best-documented compound for promoting β-cell proliferation, in various models, including INS-1E and MIN6 cells, isolated mouse islets and β-cells derived from human induced pluripotent stem cells (iPSC). (6) To investigate the efficacy of small-molecule DYRK1A kinase inhibitors in promoting β-cell proliferation, which may contribute to a better understanding of their role in insulin homeostasis. (7) To provide the proof-of-concept that small molecule-induced human β-cell proliferation *via* DYRK1A inhibition is achievable, which may have significant implications for regenerative medicine in the treatment of type 1 and type 2 diabetes.

This study generates crucial information to demonstrate the importance of the DYRK1A inhibitor in diabetes. The developed DYRK1A inhibitors have a remarkable ability to increase β-cell proliferation and balance blood glucose levels.

Previous research has shown that inhibiting DYRK1A has a beneficial impact on β-cell growth of β-cells and insulin production. However, the most effective small-molecule inhibitor now accessible, harmine, is distinguished by less than ideal kinase selectivity. AC27 exerted dual effects by both suppressing DYRK1A activity and causing a significant reduction in DYRK1A levels within cells, at the same time, it shows better selectivity towards DYRK1A. This was linked to an increased rate of growth of β-cells treated with inhibitors. Additionally, AC27 enhanced insulin and C-peptide production in response to glucose challenge without substantially affecting the level of glucagon. The observed results were statistically significant, resulting in a greater than 60% increase in insulin expression. The impact of inhibiting DYRK1A with AC27 was intensified by concomitant suppression of TGF-β. Crucially, the findings found in insulinoma cell lines were replicated in more sophisticated pancreatic islet models. AC27 markedly enhanced glucose-stimulated insulin production in β-cell organoids produced from induced pluripotent stem cells (iPSCs) and in isolated pancreatic islets from mice. Presented findings provide compelling evidence that the use of AC27 to inhibit DYRK1A effectively enhances the growth and activity of β-cells. This effect is particularly notable when compared to harmine, mainly due to AC27's greater ability to selectively target certain kinases.

The next section of the thesis focuses on examining the effects of leucettines on DYRK1A kinase inhibition and their efficacy in the treatment of diabetes. Leucettines, which are natural derivatives, can stimulate insulin secretion in insulinoma cells, hiPSC-derived β islets, and in vitro cultured islets of the pancreas from mice. Treatment of MIN6 and INS1E cells with leucettine reduces the GSIS impairment. In addition, leucettine L41 supplemented with LY364947 significantly enhances insulin secretion. Comparable promoting effects were noted in mouse and be-β-cell islets derived from hiPSC. Research indicates that this particular combination of drugs enhances the rate of proliferation and glucose response. This suggests that these drugs may have an impact on signaling pathways that facilitate β-cell replication, insulin biosynthesis, and secretion.

In the next studies, it was shown that pluronic increases the bioavailability of AC27 compared to other preparations (CrEL) in vivo, resulting in long-lasting normoglycemia in mice with induced diabetes. At the same time, by using poloxamer, it was possible to reduce the dose administered to mice to 10 mg/kg BW. The wide range of PPO and PEO combinations in Pluronic designs provide a diverse range of poloxamer properties and enable the development of a tailored drug delivery system. The shorter the PEO group, the more likely it is that Pluronic will behave like an oil and form an emulsion. Pluronic P123 offers stable formulations, capable

of forming micelles at low concentration levels. When compared to the untreated group, morphometric analysis of pancreatic sections treated with the test compounds revealed improved morphology and increased insulin after treatment. Moreover, AC27@P123 also showed increased effects on insulin secretion in hiPSC-derived β-cell organoids. Overall, obteined findings provide compelling evidence that DYRK1A inhibition with AC27 enhances the function and proliferation of β-cells.

Last section is focused on identifying a novel molecular target, the SPOP protein, which reduces the accumulation of PDX1 protein in adult β-cells, subsequently leading to reduced expression of key genes, such as insulin, that are crucial for maintaining β-cell mass and function. PDX1, also known as IPF1, plays a key role in the development and function of pancreatic β-cells, making it a promising target for diabetes treatment strategies. As a master regulator of insulin gene transcription, PDX1 is essential to maintain normal β-cell function, including its ability to synthesise and secrete insulin. Obtained results support the connection between SPOP modulation and the increase in PDX1 and insulin that follows, which is consistent with previous research. The conclusions are reinforced by this analysis of the observed results, which shows a consistent relationship between the treatment, changes in SPOP and insulin levels, and the resulting effects on PDX1 and insulin synthesis. The discovery of this novel regulatory pathway for PDX1 and the validation of its role suggest that therapeutic strategies aimed at modulating PDX1 turnover through SPOP could elevate PDX1 protein levels, thereby enhancing β-cell survival and functionality in diabetes treatment.

In summary, the present dissertation is in line with the current research trends that center on analyzing novel small-molecule DYRK1A kinase inhibitors and employing pancreatic β-cell organoids in diabetes studies.