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Development of FGF1 protein variants with potential application in the treatment of type II diabetes

ABSTRACT

According to the International Diabetes Federation's 2021 report, nearly 540 million people worldwide suffer from diabetes. It is the first non-infectious disease to reach pandemic status in the 21st century. This is due to the extremely rapid growth in the number of people affected by the disease. It is estimated that the number of diabetics will rise to 800 million in the next 20 years. Type 2 diabetes, driven by obesity, accounts for around 90% of all cases of the disease and is associated with insulin resistance. Despite significant advances in medicine, diabetes and its complications are still not fully controlled, and current therapies have a number of side effects, such as weight gain, decreased bone density and disruption of the body's calcium and phosphate homeostasis. This is why the search for new therapeutic solutions is so important. Fibroblast growth factor 1 (FGF1) is proposed to be a new drug candidate for the treatment of type 2 diabetes. This protein not only significantly lowers blood glucose levels in an animal model of diabetes, but also lacks adverse effects and does not cause hypoglycemia. One of the main contraindications for its use as an antidiabetic drug seems to be its high mitogenic potential.

The aim of this dissertation was to obtain a modified human fibroblast growth factor (FGF1) that retains antidiabetic properties with reduced proliferative activity and could serve as an alternative therapy for patients suffering from type 2 diabetes.

In the first stage of the project, 38 recombinant FGF1 variants were obtained. The strategy for designing the mutants included introducing substitutions to the protein sequence to lower the affinity for the FGF receptor, weaken binding to heparin, increase protein stability, and verify the importance of FGF1 interaction with integrin αVβ3 and FGF1 phosphorylation for its metabolic activity. In addition, variants of FGF1 described in the literature were also tested. Selected substitutions were also combined in multiple mutational variants. The proteins were efficiently overproduced, purified, and their identity and native state were confirmed, ultimately yielding preparations of greater than 95% purity. Subsequently, their *in vitro* biological characterization was carried out, including analysis of mitogenic activity and activation of major FGFR-dependent signaling pathways, as well as glucose uptake studies. Then, selected muteins were biophysically characterized by determining their thermodynamic parameters and affinity for the FGFR1c. The next step of the project involved testing the selected

mutational variants in a mouse model of diabetes, *db/db*. Based on the results obtained in the *in vitro* assays, 15 proteins were selected for *in vivo* testing. The proteins were administered subcutaneously and blood glucose levels and body weight of the mice were analyzed for seven days. This allowed the selection of two muteins that met the objectives of the project, showing reduced mitogenicity while maintaining antidiabetic properties. The final stage of the project involved an attempt to elucidate the mechanism of anti-diabetic action of FGF1, which has not yet been described. Using protein engineering and molecular biology techniques, it was possible to identify a new protein partner of the FGF1:FGFR1 complex, crucial for the metabolic activity of the FGF1 protein.