1. Research project objectives/Research hypothesis

Research hypothesis imply that ALKBH proteins except repairing alkylating lesions in DNA and RNA and fulfilling different biological functions, also take a part in obesity and type 2 diabetes (T2D) development. Primary aim of the study is to determine the ALKBHs role in the development of mentioned civilization diseases and investigation the regulatory mechanisms of their action on porcine model. This model is much closer to human than generally used in ALKBH studies mice model.

2. Research project methodology

Project objective:

To study the role of ALKBH proteins in obesity and T2D development, pig model will be used. Previously, we obtained and validated several porcine models, namely: 1) the neonate model with high predisposition to obesity and T2D development (IUGR infants) 2) obesity pig model without clinical signs of T2D obtained by high energy intake; 3) model of developed obesity with advanced symptoms and stable insulin resistance, and 4) the pattern of symptomatic reversion of T2D and obesity after bariatric surgery (Scopinaro method); 5) model for genetic background of obesity and T2D with the use of adult IUGR. Using several models we will be able to answer the questions concerning universal mechanisms involved in obesity and T2D and the role of ALKBH proteins in these phenomenon.

Project methodology

Biological materials of porcine origin will be analyzed to evaluate ALKBH1-8 and FTO expression in organs and tissues important for obesity development. Tissues: muscles, liver, pancreas, white adipose tissue, arenal gland and thyroid taken from mention above pig variant will be analyzed with the use of Western blot and in-tissue cytomerty. Using mass spectrometry, we plan to analyze whole proteom of selected tissues. Bioinformatic analysis will allow to select possible changes with an eventual role of ALKBH protein/proteins in obesity/T2D development. Predicted mechanisms will be confirmed with the use of western blot, and visualization of coexpression using in-tissue cytometry and confocal microscopy and also by molecular biology methods such as co-immunoprecipitation, microscale thermophoresis, two-hybrid screening system or differential scanning fluorimetry (DSF) on proteins purified in *E.coli* and/or baculovirus system of expression.

3. Expected impact of the research project on the development of science, civilization, society

The role of AlkB protein in bacteria *E.coli* (EcAlkB) has been recognized at the beginning of XXI c. It has been found that EcAlkB repairs alkylating lesions in DNA and RNA. Subsequently, homologs of EcAlkB have been discovered in almost all organisms including nine human homologs (ALKBH1-8 i FTO). These homologs, except repairing alkylation lesions in DNA and RNA, show other biological functions, also in cancer metabolism (our own results). Finding the involvement of FTO protein in obesity development has been of great importance. The newest findings imply that rather same polymorphisms in *Fto* gene create obese phenotype. However, our preliminary results strongly suggest involvement of FTO protein in obesity development and what's more, connection of FTO with T2D phenomenon. It also seems that other ALKBH proteins (homologes 1, 5, 7) take a part in functioning of particular metabolic pathways. Using pig model is a great value by itself since in comparison to generally used, not perfect, mice model, pig model can be extrapolated fully on human. Obtained results are expected to improve our knowledge on mechanisms of insulin resistance in obesity developed by environmental as well as genetic factors. Additionally, we find out tissue distribution of ALKBH, recognize new functions of mentioned ALKBH dioxygenases which significantly widen the area of our knowledge**.**