課題番号 01A(追)－7

IAMS Project Report

Principal Investigator: Ilir Mehmeti

Institute of Clinical Biochemistry, Hannover Medical School
Carl-Neuberg-Str. 1, 30625 Hannover / Germany
+49 511 532 6525
+49 511 532 6526
Mehmeti.Ilir@mh-hannover.de

"Understanding the role of oxidative and ER stress in Pancreatic β cells"

Research summary 2019

Dietary habits can have a major impact on health and may cause obesity and type 2 diabetes mellitus (T2DM). Increased circulating levels of saturated fatty acids (FFAs) and glucose are considered to be major mediators of β-cell dysfunction and death in T2DM. Although it has been proposed that endoplasmic reticulum (ER) and oxidative stress play a crucial role in gluco/lipotoxicity, their interplay and relative contribution to β-cell dysfunction and apoptosis has not been fully elucidated. To investigate this issue, we examined the toxicity of free fatty acid (FFA) compositions mirroring the FFA profiles of various popular edible oils in human EndoC-βH1 β-cells. For this purpose, we made compositions consisting exclusively of various FFAs in different volumetric percentages mimicking these oils and additionally mixtures of these compositions. Human EndoC-βH1 β-cells were incubated with different oil compositions and the toxicity, lipid droplet formation, ER-stress, and H_2O_2 production were analyzed. Compositions with prominent content of saturated as well as unsaturated long-chain FFAs showed moderate but significant toxicity both in human EndoC-βH1 β-cells and rat islets, however, without further measurable metabolic impairments. On the other hand compositions with high content of medium-chain FFAs revealed no toxicity. A composition with 50% of the very long-chain unsaturated FFA erucic acid caused high toxicity with concomitant peroxisomal H_2O_2 production. The toxicity of FFAs to human EndoC-βH1 β-cells was dampened in mixtures of FFA compositions with a significant content of medium-chain FFAs, but not with a significant proportion of unsaturated FFAs.

Research plan for 2020

Professor Oyadomari's group have established mouse β-cells lacking a transcription factor Activating transcription factor 4 (ATF4) by CRISPR/Cas9 gene editing system. ATF4, a member of the ATF/CREB family, plays a major role in regulating genes that are involved in amino acid metabolism and redox homeostasis under ER stress condition. As we obtained the ATF4 knockout MIN6 cells from Professor Oyadomari, we will characterize the role of ATF4 on mechanism of lipotoxicity to pancreatic β-cells. The following analysis will be conducted in 2020.

Insulin biosynthesis and secretion

- - Quantification of insulin secretion and also insulin content (RIA)
- - Quantification of pro-insulin (ELISA and immunohistochemistry)
- - Oxidative protein folding (Live cell imaging)

ROS and enzymes detection assays

- - General ROS quantification (H_2-DCFDA)
- - Specific superoxide (MitoSOX), hydroxyl radical (HPF, oxyDNA by FACS, Microscopy) and organelle-specific H_2O_2 quantification (HyPer, Reader, Microscopy)
- - Quantification of antioxidant enzyme activity (SOD, GPx, and catalase) and glutathione reductase activity (Spectroscopy)
- - Quantification of Nerf2, and AP1 promoter activity
- - Quantification of GSH and GSSG

Cell death – Apoptosis detection assays

- - Initiator caspases (caspase-8, -9 and -12) and effector caspases (caspase-3)
- - DNA-fragmentation / damage: Propidium iodide / oxyDNA
- - Cardiolipin peroxidation: 10-N-nonyl acridine orange (NAO)
- - Mitochondrial: Mitochondrial membrane potential (JC-1)
- - Release of cytochrome c and SMAC/DIABLO (cell fractionation, WB)