PROJECT 2

DoS: Lorenzo Piemonti

Title: Wolfram disease iPS cell modeling to study pathophysiological mechanisms leading to β cell dysfunctions

Curriculum: PhD PROGRAM IN EXPERIMENTAL AND CLINICAL MEDICINE

Link to OSR/UniSR personal page: http://www.hsr.it/ricerca/divisioni-centri-istituti-e-programmi-di-ricerca/istituto-di-ricerca-sul-diabete-hsr-dri/lorenzo-piemonti/ e http://dri.hsr.it/

Project description (*Number of characters*, *including spaces*: 2.000 - 3.000):
Wolfram syndrome type 1 is an autosomal recessive disorder mainly characterized by association of childhood-onset insulin-dependent diabetes mellitus, diabetes insipidus, hearing loss, optic nerve atrophy and progressive neurodegeneration. Disease etiology has been related to homozygous mutations of Wolfram syndrome 1 (WFS1) gene located on 4p16 and encoding an ER membrane protein named wolframin. To date, no hot spots have been identified inside gene and most of 170 bi-allelic mutations of WFS1 are uniformly distributed throughout its coding sequence. WFS1 is highly enriched in human islets, as well as in heart, lung and brain. Specifically, in pancreatic β cells, loss of WFS1 results in reduced granules acidification, thus affecting insulin processing and glucose-stimulated secretion. Patients with WS show a selective death of β cells, supposedly due to gradual increase of ER stress levels in these cells and possibly other mechanisms yet to be revealed. Over the last decade, several works highlighted a strictly relationship between ER stress and onset of type 1 and type 2 diabetes. By taking advantage of recently-developed induced Pluripotent Stem Cells (iPSC) technology and CRISPR/Cas9-mediated gene editing system, study of WS would then open to new therapeutic chances and could represent an ideal model to explore underlying causes of multifactorial diabetes.

We plan to:
- characterize WFS1 gene mutation carried by patients, exploring its familial segregation;
- generate iPSC from WS patients with mono-allelic and bi-allelic WFS1 gene mutations (and their healthy related as control) and differentiate them into β cell;
- understand impact of WFS1 loss of function on insulin translation and processing, granule exocytosis and cell death, in terminally differentiated β cell;
- uncover putative involvement of ER stress control system alterations and potential biomarkers of β cell ER stress, in order to develop a screening system for early detection of diabetes;
- improve therapeutic strategies for treatment of WS and other forms of diabetes mellitus (type 1, type 2 and monogenic), by targeting ER stress molecules to prevent progression of β cells loss and insulin resistance;
- restore wild type WFS1 gene in WS patient iPSC, in order to produce functional β cells (as well as other affected tissues) with the prospect of patient-specific cell therapy.

Skills to be acquired by the student: Tissue and cell culture; Flow cytometry; PCR; iPSC derivation and maintenance; Gene editing; Western Blot; Gel Electrophoresis; Microscopy; Statistical analysis.

References (max. 3)