PROJECT 1

DoS: Massimo ALESSIO

Title: **Brain targeting by liver-directed ceruloplasmin gene replacement therapy in the rare disease aceruloplasminemia**

Curriculum: Neuroscience and Experimental Neurology

Link to OSR/UniSR personal page: [https://research-hsr-it.sanraffaele.idm.oclc.org/en/centers/omics-sciences/proteome-biochemistry.html](https://research-hsr-it.sanraffaele.idm.oclc.org/en/centers/omics-sciences/proteome-biochemistry.html)

**Project description (Number of characters, including spaces: 2.000 - 3.000):**

Aceruloplasminemia (Acp) is a rare disease caused by mutations in ceruloplasmin (Cp) gene that result in loss of Cp ferroxidase activity fostering iron deposition in liver, pancreas and brain (Miyajima, 2015). Iron accumulation induces neurological symptoms preceded by diabetes, and anemia. Thus, Acp is part of the neurodegeneration with brain iron accumulation syndromes. Current therapies are ineffective on Acp’s neurodegeneration and a treatment is needed, also considering the exploitable therapeutic window of about 10 years from the onset of systemic symptoms to the neurological ones.

In the CpKO mouse model of Acp we demonstrated the therapeutic potential of peripheral Cp administration that, crossing the brain-barrier-systems, enters in the brain restoring the ferroxidase activity, reducing iron deposition and neuronal loss, and promoting an amelioration of the neurological symptoms (Zanardi, 2018). The Cp-enzyme replacement therapy (ERT) is a promising strategy, however it suffers of several limitations (high costs, requirement for life-long administration, patients compliance).

The project is aimed to study the therapeutic potential of stable endogenous Cp production obtained by liver-directed Cp-gene transfer in the Acp preclinical model, as strategy to bypass the ERT limits.

The hypothesis is that in Acp a brain barrier defect is present, which allows to the Cp to enter the brain, and, since the liver is the major physiological source of Cp, a liver-directed Cp gene replacement will be used to establish an endogenous production of Cp which is secreted into the bloodstream for widespread therapeutic activity, also in the brain. The goal is to reduce both systemic and neurological pathology in Acp.

To this aim, lentiviral vectors (LVs) carrying the human Cp transgene into a hepatocyte-specific expression cassette will be generated, and the best expression conditions will be set up both in cellular models, and in CpKO mice. Then the LVs will be intravenously administered for therapeutic purposes to CpKO mice at 6 months of age, when neurological signs are still absent. Cp expression will be monitored and after 4 months the therapeutic efficacy will be evaluated examining motor coordination ability, hematologic parameters, anti-Cp antibodies, the presence of Cp and ferroxidase activity in the brain, iron deposition and neuronal loss. To analyse the biodistribution of circulating Cp and to confirm the presence of brain barriers defect in Acp, labelled Cp intravenous administered to CpKO and wild type mice, will be monitored by PET imaging and tissues autoradiography.
Skills to be acquired by the student:

The candidate will acquire skills on animal model handling and behaviour tests, molecular biology (lentiviral vector production), cell biology (cell culture, virus production and cell infection), protein biochemistry (SDS-PAGE and western blot, enzymatic activity, ELISA, protein radiolabelling), histology and histochemistry (iron and lipid detection), animal PET imaging analysis and organs autoradiography.

References (max. 3)
