PROGETTO

Supervisore:	MANUNTA PAOLO
Titolo/*Title*:	Caratterizzazione del modello murino *Lss* knock-in per l'ipertensione sodio-sensibile: identificazione di nuovi meccanismi di danno d'organo associati all'ipertensione e all'invecchiamento / *Characterization of the Lss knock-in mouse model of salt-sensitive hypertension: unravelling novel pathways of organ damage associated with hypertension and aging.*
Curriculum:	Biologia Cellulare e Molecolare
Link alla pagina personale del sito web di Ateneo o del polo ospedaliero di riferimento:	https://www.unisr.it/docenti/m/manunta-paolo; https://research.hsr.it/en/divisions/genetics-and-cell-biology/genomics-of-renal-diseases-and-hypertension/paolo-manunta.html

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

<u>Background/gap of knowledge</u>

Salt-sensitive hypertension (SS-HT) and Acute Kidney injury (AKI) represent two renal pathologic conditions influenced by genetic predisposition that could possibly evolve in end-stage kidney failure. In patients; both diseases are associated with high levels of circulating endogenous ouabain, a cardiotonic hormone (1) that regulates the expression of Na^+/K^+-ATPase and activates Src pathway related to organ damage (2). Our research group has identified a genetic variant (rs2254524) in Lanosterol Synthase (*LSS*), an enzyme involved in steroids/cholesterol synthesis (3), as one of the possible factors contributing to both salt-sensitive hypertension and AKI (4). Recently, our team has developed the knock-in (KI) mouse model carrying the human *LSS* variant that has been shown to be hypertensive when subjected to a high-sodium diet.

<u>Rationale and hypothesis</u>

The current objective is to investigate the pathogenetic role of the *Lss* V643L variant in inducing inflammation, HT, and organ damage in aging Lss KI mice.

Objectives and specific aims

1) To identify the molecular mechanisms associated with the onset of salt-sensitive HT in Lss V643L KI mice subjected to a high-sodium diet (NaCl 4%) from the age of 6 months to establish at what age HT begins. Additionally, we aim to investigate the onset of inflammation, as a possible cause or consequence of HT and in organ damage.

2) To evaluate the effectiveness of therapy aimed at antagonizing the high-sodium diet and the gene variant effects in increasing blood pressure (BP) in Lss KI mice, by administering Rostafuroxin, a derivative of digitoxigenin, a powerful antagonist of the Na^+/K^+- ATPase. Evaluating whether the treatment prevents the development/progression of chronic organ damage.

3) To identify molecular mechanisms associated with HT and subsequent deterioration of renal function in naturally aged Lss KI and C57BL/6 WT mice (on a normosodic diet, 0.3% NaCl, or on a high-sodium diet, 4% NaCl). We will Investigate the onset of inflammation and HT during the aging process and their possible contribution to the process of organ damage.

4) To monitor the development or progression of hypertensive kidney disease and acute or chronic kidney injury after ischemia-reperfusion procedure.

Expected outcomes

Using this mouse model we aim to elucidate the molecular mechanisms that determine the onset and the development of hypertensive disease and organ damage in patients carrying the *LSS* rs2254524 (V642L) variant. Furthermore, the use of pharmacological treatments will allow to understand whether it is possible to prevent the development of HT and progression of tissue damage. We expect that the pharmacological therapies will be able to restore BP to normal levels in hypertensive mice, consequently improving renal outcome. Through the study of proteomics or metabolomics it will be possible to identify cellular mechanisms activated during aging and the onset of organ damage.

Competenze che deve acquisire lo studente (Max 600 caratteri spazi inclusi):

- Handling and management of mice colonies, treatment with different salt diets, and pharmacological drug

- BP measurement with tail-cuff method using the BP-2000 instrument by Visitech Systems, Inc
- Use of metabolic cages for urine collections
- Nucleic acids/proteins extraction, expression quantification
- Identification of inflammation markers in plasma, urine and tissues
- Acute Kidney Injury (AKI) induction via bilateral Ischemia-Reperfusion
- Statistical analysis of the results
- Problem solving and possible resetting of the project
- Results dissemination (writing/oral communications)

Bibliografia (max. 15)

1. Hamlyn JM, et al. Identification and characterization of a ouabain-like compound from human plasma. Proc Natl Acad Sci U S A 1991; 88: 6259-63

2. M. Ferrandi I, et al. Organ hypertrophic signaling within caveolae membrane subdomains triggered by ouabain and antagonized by PST 2238. J. Biol. Chem. 279, 33306–33314 (2004).

3 Lanzani C, et al. Adducin- and Ouabain-related gene variants predict the antihypertensive activity of rostafuroxin, part 2: clinical studies. Sci Transl Med 2010 Nov 24;2(59):59ra87

4. Iatrino R, et al. Lanosterol Synthase Genetic Variants, Endogenous Ouabain, and Both Acute and Chronic Kidney Injury. Am J Kidney Dis. Am J Kidney Dis. 2019 Apr;73(4):504-512