PROGETTO

Supervisore::	LAURA SILVESTRI
Titolo/*Title*:	**Deciphering the role of the BMP-SMAD signaling pathway modulator kielin/chordin-like protein (KCP) under physiological and pathological conditions**
Curriculum:	Biologia Cellulare e Molecolare
Link alla pagina personale del sito web di Ateneo o del polo ospedaliero di riferimento:	**https://research.hsr.it/en/divisions/genetics-and-cell-biology/regulation-of-iron-metabolism/laura-silvestri.html**

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge

The liver peptide hormone hepcidin is the master regulator of iron metabolism. It is a BMP-SMAD target gene whose activation is mediated by BMP ligands, the type I receptors, and the constitutively active type II receptors (1). The activity of this complex is tuned by hemojuvelin (HJV) (2). Mutations in HJV cause Juvenile Hemochromatosis Type 2A (JH-2A), a severe form of iron overload due to extremely low hepcidin production that leads to severe iron overload and organ failure in the first decades of life. The only therapeutic option is phlebotomy, a symptomatic approach that does not correct but even worsen hepcidin deficiency. Unlike other forms of hemochromatosis, JH-2A patients cannot benefit from new drugs based on the targeting of the hepcidin inhibitor TMPRSS6, being HJV the substrate of TMPRSS6, which is ineffective in the absence of a functional BMP coreceptor. Therefore, a therapeutic strategy that increases hepcidin in JH-2A patients is currently an unmet clinical need.

Rationale and hypothesis

The Tdark KCP is a secreted protein that enhances BMP-SMAD signaling by promoting the interaction of BMPs with BMP type I receptors (3). Although structurally distinct, the functions of KCP and HJV in enhancing ligand-mediated BMP-SMAD signaling appear to overlap. Indeed, both proteins can interact with BMP ligands and with the type I receptor ALK3. However, the role

of KCP in regulating hepcidin expression and iron metabolism and the precise mechanisms controlling its function in the liver remain unknown.

The main hypothesis of the project is that the Tdark kielin/chordin like protein (KCP) is a novel regulator of hepcidin and a therapeutic target for severe and rare juvenile hemochromatosis type 2A characterized by mutations in HJV gene.

Objectives and specific aims

Our preliminary data show that Kcp expression is reduce in severe hemochromatosis, potentially contributing to exacerbate hepcidin downregulation and iron overload.

Thus, specific aims of the project are:

Aim 1. To elucidate the mechanism and the liver cell type involved in KCP-mediated hepcidin modulation, and characterize the role of iron in modulating KCP expression.

Aim 2. To characterize the role of Kcp in modulating iron metabolism in physiological setting and disease conditions, and identify a EMA approved drug for Kcp targeting in the JH-2A mouse model.

Expected outcomes

We will show that KCP contributes to hepcidin activation under both physiological and pathological conditions and that its overexpression can increase hepcidin production. We will also generate a "mini-KCP" capable of increasing hepcidin by identifying the minimal KCP protein sequence that retains its biological activity. In parallel, drug screening will be performed to select compounds that, by increasing KCP, will upregulate hepcidin expression in JH-2A models.

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

1. In vitro studies (generation of recombinant proteins, functional assays,…)
2. Ex vivo studies (isolation of liver primary cells; coculture system to study the crosstalk between the different cell populations; silencing of target genes in primary cells)
3. RNA isolation, protein extraction, RT-qPCR, WB analysis histochemistry, ELISA,…
4. In vivo studies (colony maintenance, breeding, getotyping, mouse treatment, organ-tissues isolation, phenotype characterization)
5. Presentation of project results at national-international meetings
6. Paper writing

Bibliografia (max. 15)

1. Silvestri L, Nai A, Dulja A, Pagani A. Hepcidin and the BMP-SMAD pathway: An unexpected liaison. Vitam Horm. 2019;110:71-99. doi: 10.1016/bs.vh.2019.01.004. Epub 2019 Feb 10. PMID: 30798817.

2. Healey EG, Bishop B, Elegheert J, Bell CH, Padilla-Parra S, Siebold C. Repulsive guidance molecule is a structural bridge between neogenin and bone morphogenetic protein. Nat Struct Mol Biol. 2015 Jun;22(6):458-65. doi: 10.1038/nsmb.3016. Epub 2015 May 4. PMID: 25938661; PMCID: PMC4456160.

3. Lin J, Patel SR, Cheng X, Cho EA, Levitan I, Ullenbruch M, Phan SH, Park JM, Dressler GR. Kielin/chordin-like protein, a novel enhancer of BMP signaling, attenuates renal fibrotic disease. Nat Med. 2005 Apr;11(4):387-93. doi: 10.1038/nm1217. Epub 2005 Mar 27. PMID: 15793581.