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ERA Long-Term Research Fellowship Project

EURECA-M & CKD-MBD

Project's key info

Title of the project	**Crosstalk between vascular vitamin D signalling and bone homeostasis in CKD. Implications in mineral metabolism**
Working Group involved in the project	European Renal and Cardiovascular Medicine Working Group (EuReCa-M) & Chronic Kidney Disease – Mineral and Bone Disorder Working Group (CKD-MBD)
Principal Investigator(s) of the project	Jose Manuel Valdivielso (Spain) Juan Miguel Díaz Tocados (Spain)
Duration	12 months
Fellowship Grant	29.750,00 €
Start of the fellowship	Within 6 months after notification of the grant award to the fellow.

Receiving Institute

Name of receiving institute	Biomedical Research Institute of Lleida (IRBLleida)
Supervisor's name	Jose Manuel Valdivielso (Spain) Juan Miguel Díaz Tocados (Spain)
Supervisor's e-mail address	valdivielso@irblleida.cat jmdiaz@irblleida.cat

Project's detailed description

Project description
A direct relationship between bone health and the cardiovascular system has been observed in chronic kidney disease (CKD) patients. Notably, vascular calcification commonly accompanies renal osteodystrophy, indicating that concomitant factors can influence each other. Thus, the principal circulating factors that promote vascular calcification should be likely involved in the alterations of bone homeostasis. In the last years, some studies have proposed that calcified vascular smooth muscle cells (VSMCs) release cytokines capable of reaching bone cells (particularly osteoblasts) inducing alterations in bone remodelling; however, these actions have not been demonstrated in the CKD context so far. Recent studies in patients with atherosclerosis suggest a beneficial effect of sclerostin expression in VSMC, retaining the contractile phenotype and preventing vascular calcification. These results have particular interest in the CKD context, since the high plasma sclerostin levels have been associated with reduced mortality in CKD patients. Moreover, plasma and vascular sclerostin levels increase as vascular calcification progresses, which could suggest a possible defence response. Therefore, these studies

support the key role of VSMC-produced factors, such as sclerostin, in the prevention of vascular calcification and cardiovascular disease in CKD patients; however, whether vascular production of sclerostin could impact bone and mineral metabolism homeostasis has not been investigated.

In IRBLleida laboratory, researchers have generated a mouse model carrying a tamoxifen-inducible cre activity driven by the Myh11 promotor and the exon 4 of the VDR gene flanked by two LoxP sites (VSMC-VDR-cKO), which results in VDR specific deletion in VSMC after tamoxifen administration. Noteworthy, VSMC-VDR-cKO mice undergoing 5/6Nx and receiving calcitriol overdose do not develop vascular calcification despite the increased serum phosphate levels, which were similar to those in matched VDR wild-type controls with notable vascular calcification. In these VSMC-VDR-cKO mice with renal insufficiency, vascular expression of sclerostin was up-regulated at mRNA and protein levels, which may substantially contribute to the prevention of calcification, suggesting the existence of a vascular link between Vitamin-D-sclerostin calcification. Nevertheless, whether the vascular production of sclerostin is sufficient to increase its circulating levels and affect bone homeostasis should be investigated. Considering these relevant observations, the VSMC-VDR-cKO mouse model should be also appropriate to explore whether calcified VSMCs promote bone alterations in CKD independently of the circulating phosphorus levels and determine whether VSMC-secreted factors could impact bone and mineral homeostasis in CKD. We hypothesize that targeted ablation of VDR in the VMSCs results in the secretion of vascular cytokines capable of influencing bone cell activity and whole-body mineral homeostasis in CKD.
The objective of this project is to investigate the potential role of vitamin D signalling in VSMC on the regulation of bone and mineral metabolism and the progression of cardiovascular disease in CKD. Results from these studies will have great value in the context of CKD, providing new knowledge on the connection among mineral metabolism, cardiovascular system and bone health and highlighting the direct impact of vascular therapies in bone and mineral homeostasis.

All the studies in this fellowship will be developed at the Biomedical Research Institute of Lleida (IRBLleida)- Vascular and Renal Translational Research Group. The methodological approach of this proposal is divided into the 3 work packages (WPs):

- WP1: To study the direct effects of conditioned medium from VSMC deficient in vitamin D receptor on osteoblastic differentiation.
- WP2: To study whether ablation of Vitamin D signalling in VSMC produces significant effects on bone histomorphometry in a mouse model of CKD-associated vascular calcification.
- WP3: To investigate whether disruption of the vitamin-D signalling in VSMC impacts mineral metabolism by targeting bone-derived hormones in CKD conditions.

Goals of the project

The project aims to:

- evaluate the direct impact of the vitamin D signalling in VSMCs on the production of secreted factors affecting osteoblast-like cells in vitro;
- study whether targeted ablation of vitamin D signalling in VSMCs modifies bone

histomorphometry and affects cardiovascular features in a mouse model of CKD-induced vascular calcification;
- determine whether sclerostin production by VSMCs lacking VDR is capable to target bone cells and alter endocrine regulation of mineral metabolism.

Qualifications and/or expertise required to the fellow

Qualifications of the candidate that are required for successful execution of the project are:

- Concluded PhD in science when the fellowship begins.
- Qualification for development and management of transgenic animal models, as well as previous experience with mouse models of CKD.
- Background on *in vitro* models of primary culture and cell lines, preferably with vessel smooth muscle cells and osteoblastic cell lines and primary cultures.
- Expertise in performing biochemical analyses such as western blot, immunohistochemistry, quantitative PCR, ELISA, colourimetric assays, etc.
- It would desirable previous experience in bone analyses.