UNIVERSITE BIOLOGIE BRETAGNE SANTE LOIRE

Year 20 /20

THESIS TOPIC

Subject N° (to be completed by the ED):	**FUNDING:** ☒ **Requested** ☐ **Acquired**	**Funding origin:**
Thesis title: **Role of primary cilia associated to extracellular vesicles in vascular and metabolic diseases**		3 keywords: Extracellular Vesicles Primary cilia Diabesity
Unit / team: **SOPAM INSERM 1063 Angers**		
Supervisor's name: **Martinez Carmen**		Phone number: +33 2 44 68 85 79 Email address: carmen.martinez@univ-angers.fr

Socio-economic and scientific context (approximately 10 lines):
Primary cilia are organelles that protrude from most mammalian cells and play a crucial role in detecting signals critical to cell proliferation and differentiation. Thus, they dynamically participate in the coordination of cellular behaviors, in development and health. Centriolar satellites are small granular structures tethered to the scaffold protein pericentriolar material 1 (PCM1), which gravitate toward the centrosome and orchestrate ciliogenesis. Mutations in some genes encoding for centriolar satellites components lead to defects in primary cilia, and cause a wide set of overlapping human syndromes called ciliopathies. Of note, PCM1 and centriolar satellites have been recently linked to physiological function of endothelial cells and adipocytes. Removal of endothelial cilia favors atherosclerosis in ApoE-/- mice fed with a high-fat, high-cholesterol diet. Also, in mice, high-fat diet causes severe defects of primary cilia in visceral adipose tissue leading to adipose tissue expansion. Yet, how the impairment of primary cilia leads to cardiovascular and/or metabolic disorders remain poorly understood. Lately, it has been suggested that bioactive extracellular vesicles can be released from the surface of the cilium. By this mechanism, cilia receive signals from the environment and transmit these signals to other parts of the cell.

Working hypothesis and aims (approximately 8 lines):
We have shown elevated levels of circulating EVs, mainly from platelets, erythrocytes and endothelial cells, in obese and/or metabolic syndrome patients. These EVs are correlated with the severity of the pathology and induce vascular dysfunctions associated with inflammation and oxidative stress through the activation of the unfolded protein response. Also, unpublished data show that, in vascular smooth muscle cells, EVs from metabolic syndrome patients evoke proliferation, migration and increase pro-inflammatory cytokine production, recapitulating the early steps of atherosclerosis. On the other hand, proteomic analysis of EVs from T cells has allowed to identify proteins associated with cilia (unpublished results). Also, we have detected acetylated tubulin, a cilia marker, in circulating EVs from patients with cardiometabolic disorders. Altogether, these data suggest a link between diabetes, obesity, vascular injury, EVs and primary cilia. Based on these data and our observations, we hypothesize that EVs from primary cilia may play a critical role in the genesis and/or progress of cardiovascular and metabolic disorders.

Main milestones of the thesis (approximately 12 lines):
- To characterize EVs (size, morphology, number and composition) from human and mouse endothelial cells and mouse adipocytes presenting cilia versus cells that have lost cilia. Primary cilia will be removed by pharmacological and siRNA approaches. Differential expression of ciliary proteins in circulating EVs from patients with metabolic disorders versus healthy subjects will be performed in order to verify whether levels of cilia EVs are modified during cardiometabolic disorders.
- To analyze the effects of EVs from cilia in cellular and animal models. This part of the project wants to determine whether cilia EVs are involved in the metabolic and cardiovascular alterations described in MetS patients. To this end, EVs will be used for the analysis of the in vitro endothelial cell and adipocyte functions by using human endothelial cells and murine adipocytes. Alterations on the permeability and nitric oxide production will be studied in endothelial cells, whereas lipid accumulation and adipocyte differentiation will analyzed in adipocytes. qRT-PCR arrays and ELISA will be used to analyze the secretome of both endothelial cells and adipocytes. Also, we will analyze the effects of cilia EVs on in vivo endothelial functions, atheroma plaque formation and lipid accumulation in liver in mouse high-fat diet models. Then, tissues will be dissected: (i) vascular reactivity will be analyzed by myography; (ii) atheroma plaque formation will be quantified; (iii) lipid accumulation in liver, and (iv) ELISA, Western blots and qRT-PCR will be used to analyze to evaluate cytokine production and signaling pathway activation

Scientific and technical skills required by the candidate (2 lines):
- Cellular Biology, Molecular biology, Imaging and Functional studies of vascular function in mice.

3 publications from the team related to the topic (last 5 years):

- Malloci M, Perdomo L, Veerasamy M, Andriantsitohaina R, Simard G, Martínez MC. Extracellular Vesicles: Mechanisms in Human Health and Disease. Antioxid Redox Signal. 2019;30:813-856

- Safiedeen Z, Rodríguez-Gómez I, Vergori L, Soleti R, Vaithilingam D, Douma I, Agouni A, Leiber D, Dubois S, Simard G, Zibara K, Andriantsitohaina R, Martínez MC. Temporal cross talk between endoplasmic reticulum and mitochondria regulates oxidative stress and mediates microparticle-induced endothelial dysfunction. Antioxid Redox Signal. 2017;26:15-27

- Vergori L, Lauret E, Gaceb A, Beauvillain C, Andriantsitohaina R, Martinez MC. PPARα regulates endothelial progenitor cell maturation and myeloid lineage differentiation through a NADPH oxidase-dependent mechanism in mice. Stem Cells. 2015;33:1292-303.

National and international collaborations:

- Pr Hermenegildo C, Physiology Department, University of Valencia, Spain

- Pr Ghaleh Bijan, INSERM U955, Créteil, France