COLLEGE DOCTORAL BRETAGNE SCIENCES DE LA VIE ET DE LA SANTE

Year 2022-2023

THESIS TOPIC

Subject N° (to be completed by the ED):	**FUNDING:** ☒ **Requested** ☐ **Acquired**	**Funding origin: Ordinary doctoral contract**
Thesis title: **Extracellular vesicles as new biomarkers of endocrine disruptors exposure in the progression of steatotic liver diseases**		3 keywords: pollutants liver Extracellular vesicles
Research Unit / team: **IRSET U1085 / team 3 « Stress, membrane and signaling**		
Director's name: **Corinne Martin-Chouly** Co-director's name : Dominique Lagadic-Gossmann Co-supervisor's name : Normand Podechard		Phone number: 0223234904 Email address: Corinne.chouly@univ-rennes.fr Year of HDR :2010

Socio-economic and scientific context (approximately 10 lines):

Our environment, from the food to the air breathed or to the objects used, contains chemical molecules that can constitute a risk or even a danger for human health. Thus, endocrine disruptors (EDs) can alter all endocrine functions that produce hormones essential to our health. Many questions are about their effects in humans and this is one of the most important public health concerns of recent years. To respond to the politic implemented by the European Union to reduce the levels of PE exposure, it is necessary to develop new tools to assess the impact of these molecules on human health. As part of the European OBERON consortium, the objective is to build a strategy of integrated tests to detect metabolic disorders linked to EDs. This project combines experimental approaches (*in vitro* and *in vivo*), high throughput omics technologies, epidemiology and human biomonitoring studies as well as advanced computer models on functional parameters related to metabolism. In this context, our team is involved in the development of *in vitro* biological assays on human hepatocytes HepaRG and *in vivo* in zebrafish to evaluate the impact of EDs in the progression of specific pathologies, i.e. NAFLD (Non-Alcoholic Liver Diseases). NAFLD include liver damage ranging from steatosis (characterized by an excessive accumulation of fat) to steatohepatitis (characterized by inflammation and cell death, which can then progress to cirrhosis and hepatocellular cancer). Although the molecular and cellular mechanisms of this transition are not fully understood, exposure to xenobiotics, including EDs, is strongly suspected to participate. Thus, there is a real urgency for regulatory agencies (OECD) to have adequate tools to assess the impact of EDs in this context. In addition, it has been demonstrated a major role of extracellular vesicles (EVs) as players in cellular communication in the pathogenesis of liver diseases. EVs are vesicles with a double membrane released by cells into the extracellular environment. Our team has developed expertise in the analysis of EVs produced by different cell types exposed to xenobiotics. The content of these EVs can have an impact on the functions of recipient cells; in addition, data from the literature indicate that this content might be used to monitor the progression of NAFLD. Thus, EVs could serve as new biomarkers of metabolic effects of EDs.

Working hypothesis and aims (approximately 8 lines):

EDs include several hundred compounds defined by their ability to interfere at any level with the action of hormones; among them, some are able to increase the susceptibility to metabolic disorders (NAFLD, diabetes, obesity). In a similar context, our team has already shown that exposure to certain xenobiotics (hydrocarbon / alcohol or phthalate / alcohol) promotes the transition from steatosis to steatohepatitis. However, it remains essential to assess more precisely the impact of EDs in NAFLD, but also to better understand the molecular and cellular mechanisms involved in the deleterious effects of these molecules. In this context, the main objectives of this thesis are: 1) the development and validation of *in vitro* approaches to evaluate the effects of EDs on NAFLD in the HepaRG model; 2) to evaluate the effects of these EDs on the production of EVs by HepaRG; 3) to test the influence of EVs from HepaRG treated with EDs on liver damage in zebrafish and macrophage culture (inflammation, cell death).

Main milestones of the thesis (approximately 12 lines):

This thesis project mainly aims at evaluating the impact of EDs in NAFLD and to determine their mechanisms of action while seeking to develop *in vitro* tests for screening the effects of metabolic disturbances of EDs on the liver using culture of human hepatocytes HepaRG. To respond to this challenge, this work will consist of 3 stages. The first step will be to evaluate the potential of EDs to induce steatosis by measuring lipid metabolism in HepaRGs (Seahorse / palmitic acid, triglyceride assay, Nile Red, EROD) and to compare the effects of EDs with fatty acids or amiodarone supplementation (known inducers of steatosis in HepaRG). In the second step, we will study the effects of these EDs on the production of EVs by HepaRG in conditions of steatosis or not (ultrastructure by electron microscopy, size and quantity by NTA (nanoparticle tracking analysis), protein content by Western blotting and cytometry, lipid content by cholesterol assay). In the third step, we will expose zebrafish or macrophages to EVs produced by steatotic HepaRG to study liver damage and inflammation (phagocytosis using kit with fluorescent bacteria; production of inflammatory markers (ELISA, qPCR); cell death).

Scientific and technical skills required by the candidate (2 lines):

The candidate must have a Master 2 in the field of Biology & Health (or equivalent), and preferably have serious experience in the field of toxicology. He should have a good general level in cell biology (lipid metabolism, intracellular trafficking, inflammation) and good skills in cell culture. He must have a high sense of organization, a strong aptitude for teamwork (interaction, coordination, proposal, dynamism) while being able to work independently, good capacities to synthesize and present scientific results (communication) and good skills in English

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

- Tête A*, Gallais I*, Imran M, **Martin-Chouly C**, Sparfel L, Bescher M, Sergent O*, **Podechard N***, **Lagadic-Gossmann D***. MEHP/ethanol co-exposure favors the death of steatotic hepatocytes, possibly through CYP4A and ADH involvement. Food Chem Toxicol, 2020. 146 : 111798.
- van Meteren N, **Lagadic-Gossmann D**, Chevanne M, Gallais I, Gobart D, Burel A, Bucher S, Grova N, Fromenty B, Appenzeller BMR, Chevance S, Gauffre F, Le Ferrec E, Sergent O. Polycyclic aromatic hydrocarbons can trigger hepatocyte release of extracellular vesicles by various mechanisms of action depending on their affinity for the aryl hydrocarbon receptor. Toxicological Sciences, 171 : 443-462, 2019.
- Le Goff M, **Lagadic-Gossmann D**, Latour R, **Podechard N**, Grova N, Gauffre F, Chevance S, Burel A, Appenzeller BMR, Ulmann L, Sergent O, Le Ferrec E. PAHs increase the production of extracellular vesicles both in vitro in endothelial cells and in vivo in urines from rats. Environmental Pollution, 255 :113171 p., 2019.

National and international collaborations:

- Department of Cytokinetics, Institute of Biophysics of the Czech Academy of Sciences, Brno, République Tchèque – Jan Vondracek (projet européen Oberon)
- RECETOX, Faculty of Science, MasarykUniversity, Kamenice, Brno, République Tchèque – Pavel Babica (projet européen Oberon)
- Instituto de Investigación, Desarrollo e Innovación en Biotecnología Sanitaria de Elche (IDiBE), Universitas Miguel Hernández, Elche, Espagne – Alonso-Magdalena Paloma (projet européen Oberon)
- STLO – Agrocampus INRAE (Université de Rennes 1) - Dr E Guédon (NTA, vésicules extracellulaires)
- UMR CNRS 6226 Institut des Sciences Chimiques de Rennes- Dr F Gauffre (NTA, vésicules extracellulaires)
- IRSET Inserm U1085 (Université de Rennes 1) – équipe 5'Dynamique du microenvironnement et cancer'– Dr Sophie Langouet (projet européen Oberon)
- Inserm UMR S-1124 "T3S, Environmental Toxicology, Therapeutic Targets, Cellular Signaling and Biomarkers", Paris – Dr Xavier Coumoul et Dr Karine Audouze (projet européen Oberon)