Research Project Proposal
Academic year 2021-2022
Máster en Investigación Biomédica

Project Nº 45

Title: Role of post-transcriptional modifications (PTMs) in the crosstalk between cholangiocarcinoma and its micro-environment

Department/ Laboratory
Liver Diseases Group,
Department of Hepatology and Gastroenterology
Biodonostia Health Research Institute (Donostia University Hospital)
Paseo del Dr. Begiristain s/n
20014 Donostia - San Sebastian

Director 1: Dr. Jesus Maria Bañales
Contact: jesus.banales@biodonostia.org
Tel: +34 627401179 (Mobile) / +34 943006067 (Office)
Codirector: Dr. Paula Olaizola Rebé
Contact: paula.olaizola@biodonostia.org
Tel: +34 616207228 (Mobile) / +34 943006125 (Office)

Summary
Cholangiocarcinoma (CCA) comprises a heterogeneous group of biliary malignant tumors characterized by dismal prognosis. Incidence is increasing worldwide and already represents the second most frequent primary liver cancer. The etiopathogenesis of CCA remains largely unknown. Therefore, there is an urgent need to unveil the mechanisms involved in its development and progression in order to find new therapeutic targets and early diagnostic/prognostic biomarkers.
CCA presents an extensive tumor microenvironment (TME), which includes immune cells, endothelial cells and cancer-associated fibroblasts (CAFs). These interact with each other and with the tumor by secreting and receiving different molecules. Recently, TME has gained interest as it can drive neoplastic transformation and regulate several cancer hallmarks. Moreover, TME has emerged as an important factor in the appearance of chemoresistance.

We have previously demonstrated that inhibition of NAE1 and UBE2I, key proteins in the NEDDylation and SUMOylation pathways, in CCA cell lines significantly reduces CCA cell line viability, proliferation, spheroid and colony forming ability *in vitro*, as well as diminishes tumor growth *in vivo*. Therefore, indicating that inhibition of NEDDylation and SUMOylation pathways can have potential therapeutic value in CCA. In this regard, we now aim to evaluate the relevance of these post-translational modifications (PTMs) in the crosstalk between the tumor and its microenvironment, by performing co-culture experiments with *NAE1* or *UBE2I* knockdown cells and CAFs, endothelial cells, or monocytes. Additionally, we will use the NAE1 and UBE2I pharmacological inhibitors on CAFs, endothelial cells, and immune cells to evaluate the effect of PTM inhibition on the crosstalk between the tumor and stroma, and ultimately on CCA progression. Our preliminary data indicate that CRISPR/Cas9-mediated knockdown cells secrete fewer proteins involved in angiogenesis, CAF activation and proliferation as well as inflammation. Thus, we hypothesize that the NEDDylation and SUMOylation pathways could represent potential targets to halt highly desmoplastic CCA growth, being potential therapeutic targets.

Aims:

1. Analysis of the NEDDylation and SUMOylation pathway components in CCA cell lines, CAFs, as well as in endothelial and immune cells.
2. Determination of the role of NAE1 and UBE2I in the crosstalk between CCA and its stroma.
3. Investigation of the potential therapeutic value of Pevonedistat and ML792, pharmacological inhibitors of the NEDDylation and SUMOylation pathways, respectively, targeting both tumor and stromal cells.

Methodology:

1. CCA and normal liver samples from 5 large international cohorts.
2. Cell culture of CCA cells, normal human cholangiocytes, CAFs, endothelial cells and immune cells. Co-culture of CCA cells with stromal cells.
3. Orthotropic tumor xenografts (mice).
4. CRISPR/Cas9 technology to silence NAE1 and UBE2I.
5. Proliferation, cell cycle and cell death assays.
6. Expression analysis (qPCR, WB, IHC).

yes	**X**
no	

Does the project include the possibility of supervised animal manipulation to complete the training for animal manipulator?