Universidad de Navarra
Facultad de Ciencias

MÁSTER EN INVESTIGACIÓN BIOMÉDICA
Research Project Proposal
Academic year 2026-2027

Project Nº 21

Title: *Functional characterization of circulating tumor DNA release dynamics in in vitro models of cholangiocarcinoma*

Department/ Laboratory ASOCIACIÓN INSTITUTO DE INVESTIGACIÓN SANITARIA BIOGIPUZKOA LIVER DISEASES GROUP

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Summary Cholangiocarcinoma (CCA) is a highly aggressive malignancy with limited tools for real-time disease monitoring and prognostication. Circulating tumor DNA (ctDNA) has emerged as a promising minimally invasive biomarker, yet its clinical implementation is hindered by a limited understanding of the biological mechanisms governing its release and its relationship with tumor behavior. Addressing this gap is essential to enable the reliable interpretation of ctDNA metrics in clinical decision-making. The main **OBJECTIVES** are: (i) to characterize ctDNA release across genetically diverse CCA cell lines under different biological conditions; and (ii) to link ctDNA dynamics with cellular phenotypes such as proliferation, apoptosis, and senescence.

A panel of human CCA cell lines, including chemoresistant derivatives, will be cultured under baseline conditions, exposure to protumorigenic stimuli (e.g., Wnt, TGFβ1, cytokines), and clinically relevant treatments. Additionally, co-culture systems with cancer-associated fibroblasts will be used to model microenvironmental influences. Cell-free DNA will be isolated from culture supernatants at multiple timepoints. ctDNA will be quantified using mutation-specific digital droplet PCR, enabling precise measurement of tumor-derived DNA. Key readouts will include ctDNA detectability, absolute mutant copies, release rates, and variant allele frequency dynamics. Parallel functional assays will assess apoptosis (TOPRO/Annexin V, caspase activity), proliferation (CFSE), and senescence (β-galactosidase), allowing mechanistic interpretation of ctDNA release patterns.

By integrating ctDNA dynamics with functional cellular states, this project will generate biologically grounded evidence to support the interpretation of liquid biopsy readouts. Ultimately, these findings will contribute to improving the clinical utility of ctDNA for patient stratification, treatment monitoring, and early detection of disease progression in CCA.

yes	X
no	

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