CHOLANGIOCYTE ORGANOIDS AS A TOOL FOR CHOLANGIOPATHIES RESEARCH

Cholangiopathies are chronic liver diseases including primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), cystic fibrosis involving the liver, biliary atresia, polycystic liver disease, and cholangiocarcinoma, that share a central target: the cholangiocyte[1]. The cholangiopathies account for substantial morbidity and mortality given their progressive nature, the challenges associated with clinical management, and the lack of effective medical therapies. Thus, cholangiopathies usually result in end-stage liver disease requiring liver transplant to extend survival.

Cholangiocytes are actively involved in the modification of bile volume and composition, are activated by interactions with endogenous and exogenous stimuli, and participate in liver injury and repair [2]. The putative pathogenic model of cholangiopathies shows an initial insult to cholangiocytes as an endogenous or exogenous substance, a microorganism, or an unidentified environmental exposure that lead to the host response characterized by a reactive cholangiocyte phenotype and a bile duct proinflammatory microenvironment[1, 2]. It generally resolves with the resolution of the insulting agent to the biliary tree. However, perpetuation of the initial inflammatory response leads to chronic inflammation of the bile ducts and ultimately to cholestasis, bile duct proliferation, ductopenia, fibrosis, and potential malignant transformation of cholangiocytes. Disorders of the biliary system account for 70% of pediatric and up to a third of adult liver transplantation [3], resulting in cholestasis and cholangitis and, frequently, in the use of biliary interventions or even retransplantation.

Cholangiocyte organoids

Acquired cholangiopathies, such as Primary Biliary Cholangitis (PBC) and Primary Sclerosing Cholangitis (PSC) are complex multifactorial diseases that are still lacking satisfactory study models[4]. A major problem in cholangiocyte research is the lack of in vitro human models to study

the pathogenesis of cholangiopathies and to validate potential therapeutic targets. While the use of animal models suffers often from a lack of phenotype reproducibility, primary human cholangiocytes are difficult to isolate, de-differentiate after a few passages. Furthermore, immortalized cell lines do not faithfully recapitulate normal physiological functions of cholangiocytes.

Conversely, organoids culture systems have emerged as a new frontier technology in liver and biliary research. Unlike to classic two-dimensional monolayer cell cultures that do not resemble intercellular cell-to-cell interaction and communication, organoids consist of organ-specific cell types that self-organize in a three-dimensional cell culture system recapitulating cellular interaction and organ architecture with remarkable fidelity. Moreover, they can be expanded indefinitely and maintained in long-term culture through serial passaging without losing their characteristics and can be cryopreserved as biobanks[5]. Organoids derived from cholangiocytes present an archetypal and clinically important system for developing proof-of-concept studies on biliary disorders in humans[6]. Their more accurate organ-like organization makes them a valuable tool for drug screening, disease modelling and therapeutic applications. Cholangiocyte organoids can be suitable for regenerative medicine applications and repair bile ductus after transplantation[7], creation of bioengineer artificial ducts that act as functional bile ducts once transplanted in liver[8].

Objective of the study

The objective of this study will be the development of a new model of cholangiocyte organoid that mimic pathologies at the organ level in particular the proinflammatory profile of cholangiopathies. These organoids will offer a new approach for studying the progressive, stepwise nature of cholangiopathy and can be potentially leveraged for drug screening.

To achieve its mission, our proposal addresses these operational objectives (O.O):

O.O 1: Development of cholangiocyte organoids

Human duct epithelial cells will be isolated by human bile and/or gallbladder will grow as cholangiocyte organoids[9]

To characterize cholangiocyte organoids, we will evaluate the expression of key biliary markers (cytokeratin 19, cytokeratin 7, Sox9 and gamma-glutamyl transferase (GGT)) by Real time PCR, as well as key cholangiocyte function such as alkaline phosphatase and GGT activity by ELISA.

O.O 2: In vitro model of proinflammatory cholangiopathy using cholangiocyte organoids

Since in cholestatic liver diseases, including PSC, cholangiocytes are exposed to increased levels of enteric microbe-derived lipopolysaccharide (LPS) through the portal venous circulation[10], we will activate cholangiocyte organoids with (LPS) to mimic a proinflammatory phenotype. Moreover, we will also treat cholangiocyte organoids with another inflammatory cytokines, as tumor necrosis factor alpha (TNF-α).

After stimulation with LPS and/or TNF-α, we will evaluate:

1) cell senescence, by analysis of β-galactosidase expression (Real time-PCR)
2) proinflammatory phenotype, evaluating the expression of adhesion molecules and proinflammatory chemo/cytokines that orchestrate the recruitment and activation of innate and adaptive immune cells that characterize activate cholangiocyte (Luminex Technology)

Future applications

As our future aims, once developed a cholangiocyte organoid platform, we would like:

1) to test the ability of cholangiocyte organoids to repair the biliary tree in a biliary injury mouse model, thereby paving the way for cell-based therapy using organoids.
2) to assess effects of new drugs on both our in vitro model of proinflammatory cholangiopathy and organoids from patient-derived samples.

References

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Budget proposal

	Category	*Costs in Euro*
Funding Requested	Laboratory equipment and consumables	3000,00
	Isolation, propagation and organoid culture (dissociation enzyme, matrigel, basal/complete medium for organoid growth)	12000,00
	Characterization of cholangiocyte organoids by RT-PCR and ELISA kit	6000,00
	Determination of Chemo/Cytokine Secretion (Luminex technology)	4000,00
Total		25.000,00