Primary sclerosing cholangitis (PSC) is a chronic biliary disorder with a complex etiology, characterized by progressive destruction of the biliary tract, and consequently the liver, through the mechanisms of autoimmunity and cholestasis. A unique feature of PSC is a concurrent diagnosis of inflammatory bowel disease (IBD) occurring in approximately 70%-80% of PSC patients, mainly ulcerative colitis (UC). The etiology of colon inflammation in PSC patients (PSC-UC) is unknown. Moreover, the presence of concomitant PSC with UC represents a distinct disease phenotype that carries a higher risk of colorectal cancer (CRC) than the average IBD patient. This risk is thought to be 4–10 times greater than the risk of developing colorectal carcinoma (CRC) in patients with UC without PSC, and develops at a much younger age than in patients with UC alone. **The issue of factors linking UC to PSC is highly under investigated, while no effective treatment modalities are available.**

Emerging evidence has shown that epigenetic modifications such as DNA methylation, processed by *DNA-methyltransferases* (DNMTs), and miRNAs-modulated gene expressions influence a development of many diseases. MicroRNA (small non-coding RNA molecules) are key players in a regulation of immune response and their expressions are dysregulated in different type of cancer. MiR-155 is known as a multifunctional regulator of naïve and adaptive immunity. Our pilot studies showed that miRNA-155, and its target genes (*sphingosine-1-phosphate receptor 1* - S1PR1, and *suppressor of cytokine signaling* - SOCS1) are inversely expressed in colon of PSC with concurrent UC (PSC-UC) vs. UC patients. Moreover, we observed the dysregulation of DNMT1 in colonic tissue of PSC. **This may suggest differences in the pathogenesis of inflammation and CRC in PSC with concurrent UC (PSC-UC) patients in comparison to patients with UC alone.**

To prove our hypothesis that miR-155 may play a role in the etiology and development of colorectal neoplasia and/or inflammation in PSC-UC we are going to carry out the following experiments:

Firstly, we aim to investigate whether the observed changes in our pilot study (n=10) will be present in an validation study. We assessed the minimum number of subjects for adequate study power, and we are going to enlarge each study group (PSC, PSC-UC, UC, control) by 20 subjects. Moreover, to obtain a broader picture on miR-155 expression in the whole spectrum of the PSC disease we are planning an inclusion of colorectal cancer samples from patients with PSC-UC (n=20). In collected colonic samples (ascending and sigmoid colons) we will examine the expression of miR-155, S1PR1, SOCS1, *interleukin 17A* (IL17A), *forkhead box P3* (FOXP3), s*ignal transducer and activator of transcription 3* (STAT3), and levels of enzymes involved in the *sphingosine-1-phosphate* (S1P) metabolism including *sphingosine kinase* (SPHK1), and *sphingosine ligase* (SPL).

Secondly, it is now widely accepted that DNA methylation plays a key role in the silencing of numerous cancer-related gene. Therefore we will determine the level of the *phosphatase and tensin homolog* (PTEN) promotor methylation in the examined colon tissue (collaboration with University in Pamplona, Spain).

Thirdly, miRNAs are present and highly stable in biofluids like plasma. Therefore, having the access to a large well-characterized group of PSC (n=300), PSC-UC patients (n=300), and gender-matched, age-matched controls (n=300), we plan to assess miRNA expressions in serum samples from those patients and investigate the association between the level of miR-155 and colonic inflammation and/or occurrence of CRC in those patients. We also plan to investigate the **relation between serum expression of miR-155 and the clinical and laboratory features** in an analyzed cohort. Additionally, based on our *in vitro* observation of induction of miR-155 expression in response 5-aminosalicylic acid treatment (5-ASA; drug used for the treatment of UC), we plan to analyze the effect of this drug in a further group of PSC patients (n=30).

Fourthly, we aim to examine the possible mechanisms of miR-155 actions that may drive an immune system imbalance or cancer development. We plan functional studies **in human intestinal epithelial cell lines,** (i) to investigate whether the inhibition or induction of miR-155 can modulate the expression of components of STAT3/S1P axis and the Th17/Treg ratio; (ii) to analyze the level of miR-155 and its target genes in cells treated with lipopolysaccharide (LPS) which induces pro-inflammatory response; (iii) to gain insight on the effects of the drugs during ongoing inflammation in the intestine the expression of miR-155 and its targets will be measured after drug exposure in LPS-stimulated the cell lines.

The analyses will be carried out in colon tissue, and serum collected from patients with PSC, PSC-UC, PSC with colorectal cancer, UC, and controls. The functional *in vitro* analysis will be examined in Caco-2, HT29, and NCM460 cells line. Expressions of specific genes will be measured using TaqMan Gene Expression Assays and Real-time PCR, and protein levels will be evaluated using Immunobloting. The visualization of miRNA molecules in frozen and formalin-fixed paraffin-embedded tissue sections will be performed using In Situ Hybridization.

We believe, that understanding of the role and modifications of miR-155 in cellular functions and colonic pathological processes in PSC provide a strong molecular basis for the development of miRNA-based therapies.