DESCRIPTION FOR THE GENERAL PUBLIC

Chronic hepatitis C (CHC) is a major public health problem and it is estimated that it affects about 130 to 170 million people of the world population. According to Flisiak et al. in Poland, estimated number of people with anti-hepatitis C virus (HCV) antibodies is about 730 000, including about 230 000 people with active HCV replication. Chronic HCV infection is considered to be a major cause of cirrhosis and hepatocellular carcinoma. Approximately 350 000 people die each year from HCV-related liver diseases. Fibrosis as an integral process and the most important complication of chronic liver disease is serious problem of clinical medicine. Currently, the "gold standard" for the diagnosis and assessment of fibrosis extent and inflammation activity in the liver is the histopathological evaluation of liver biopsy specimens. This method is invasive and not without side effects, therefore there is a need for non-invasive markers of the severity of pathological lesions in the liver.

Hitherto a few genetics factors have been identified as having pivotal role in the development and course of HCV-related liver diseases. The best documented is the polymorphisms rs738409 (C>G) of patatin-like phospholipase domain-containing protein 3 (*PNPLA3*), known also as adiponutrin. It was revealed that occurrence of genotype GG in CHC patients is associated with higher risk of advanced liver inflammation and fibrosis in these patients. Despite the fact that many studied on *PNPLA3* in pathogenesis of HCV-related liver diseases has been conducted, still the epigenetic mechanism of its regulation as well as its influence on the development of pathological lesions in liver has not been elucidated. Recently scientists' attention has been attracted by small RNA molecules called microRNAs (miRNA, miR), which are considered as one of the most important post-transcriptional regulators of gene expression. miRNA discovery launched a wave of intensive research on biological role of these molecules both in psychological and pathological conditions, including CHC. Among miRNA of pro-fibrosis activity can be mentioned, among others: miR-17-5p, miR-21, miR-181b, miR-221/222. In contrast, the opposite character is postulated for miR-101, miR-122, miR-146, miR-214 etc. Current research focuses on gaining knowledge on mechanism of their action in the context of the development and progression of liver diseases. *In silico* analyses allow to determine that PNPLA3 expression is regulated by miRNA-335-5p. The results of our independent preliminary studies have revealed significantly higher expression of miRNA-335-5p in CHC patients compared to healthy donors.

The aim of this study is to know the importance of miRNA-335-5p in post-transcriptional regulation of *PNPLA3* expression as well as to reveal the association of miR-335-5p–*PNPLA3* interaction and the development of liver fibrosis and cirrhosis in patients with CHC.

The results obtained allow to **verify the following hypotheses**:

1. There is an association between expression level of miRNA-335-5p and *PNPLA3* gene expression on the mRNA and protein level in Huh7 cell line.

2. Single nucleotide polymorphisms (SNPs) in the miRNA-335-5p binding site influence the strength of miRNA-335-5p binding, and the expression of mRNA and protein of *PNPLA3.*

3. Analysis of miRNA-335-5p expression as well as of occurrence of SNPs in the miRNA-335-5p to *PNPLA3* mRNA binding sites is useful for predicting the advancement of pathological lesions in the liver of CHC patients.

The material for the study will be venous blood, collected from 100 patients with chronic hepatitis C and 100 healthy donors. *In vitro* studies will be performed on Huh7 cell line. Evaluation of the biological role of miRNA-335-5p will include: **(1)** experimental validation of miRNA-335-59–*PNPLA3* interaction; **(2)** assessment of association between expression of miRNA-335-5p and of *PNPLA3* on the mRNA and protein level; **(3)** determination of the influence of selected SNPs occurrence on the strength of miRNA-335-5p binding to *PNPLA3* and its expression on mRNA and protein level. In the last part of the study the relationships observed *in vitro* will be verified *in vivo*, that is in group of CHC patients and healthy subjects.

Research proposed in this study have **clearly innovative character.** There are no studies confirming the importance of miRNA-335-5p in regulation of *PNPLA3* gene. No analyses evaluating the influence of miRNA-335-5p on *PNPLA3* mRNA and protein were conducted. The impact of SNPs occurrence in miRNA-335-5p binding site on the strength of its binding to *PNPLA3* mRNA has also not been studied. Moreover there are no data concerning the significance of miRNA-335-5p in the development of liver cirrhosis as well as data showing the correlation between miRNA-335-5p expression and advancement of pathological lesions in liver of CHC patients. Understanding aforementioned relationships allows to insight into mechanism underlying the development of pathological lesions in the liver. It expands current knowledge on the miRNA-335-5p role in regulation of *PNPLA3* gene as well as on the importance of miRNA-335-59–PNPLA3 interactions in development of liver fibrosis and cirrhosis in patients with CHC.