SUMMARY

Hepatitis C is a serious public health problem. It is estimated that about 71 million people are infected with hepatitis C virus (HCV) worldwide, which is about 1% of the general population. In Poland, the estimated percentage of people with anti-HCV antibodies is about 1%, of which about 200,000 (0.5%) are people with active virus replication (presence of HCV RNA). In about 80% of infected individuals, the inflammation becomes chronic (CHC) and over many years leads to progressive liver damage. There are approximately 400.000 deaths from HCV-related liver disorders each year, mainly cirrhosis and hepatocellular carcinoma. The current treatment that targets the virus directly is highly effective, however still many people are unaware of being infected and are a potential source of HCV spread. Furthermore, preventing new infections is also problematic due to the lack of an HCV vaccine.

MiRNAs are short, single-stranded, endogenous RNA molecules whose main role in living organisms is post-transcriptional regulation of gene expression. Many studies indicate that miRNAs act as messengers during virus-host interaction, modulating antiviral response and progression of liver damage during CHC. In addition, these molecules are stable and possible to detect in body fluids, and are therefore a potential source of non-invasive prognostic and predictive markers in CHC. In addition, at this point there exists little research on the biological role of miRNA in CHC as well as their impact on the HCV life cycle and infectivity. Therefore, there is a need to expand research on the interaction of miRNAs with the HCV genome and the importance of these molecules for virus elimination and disease progression. The aforementioned premises and still unclear mechanism of HCV persistance prompted to undertake studies on expression and interaction of selected miRNAs with target mRNAs in CHC.

The main goal of the doctoral dissertation was to understand the importance of selected miRNAs for the development and course of CHC. The aim was achieved by analyzing the expression of 179 selected miRNAs in the plasma of patients and assessing the impact of miR-106b-3p, miR-331-3p and miR-335-3p on the hepatocyte function and HCV replication *in vitro* in the Huh7.5 liver cell line. The interaction of the studied miRNAs with putative mRNA targets as well as their effect on HCV replication were also assessed.

The study examined the expression of 179 circulating miRNAs in patients with CHC and healthy donors. It was shown, that HCV infection significantly change the expression profile of selected miRNAs in plasma. On this basis, 5 miRNAs with significantly increased expression compared to healthy donors were selected, i.e. miR-106b-3p, miR-145-5p, miR-324-5p, miR-331-3p and miR-335-3p. There was reported a weak positive correlation between miR-145-5p expression and AST and GGTP levels, as well as miR-106b-3p and miR-331-3p and AST levels in plasma of CHC patients. However, no relationship between the expression of studied miRNAs and

plasma HCV RNA level was found. For further functional analyzes, miRNAs with the highest difference in expression between patients and healthy donors were selected, i.e.: miR-106b-3p, miR-331-3p and miR-335-3p. MiR-106b-3p has been shown to significantly reduce proliferation, slow down the cell cycle and reduce apoptosis of Huh7.5 cells. Similarly, it was reported that miR-335-3p lowers the proliferation and slows down the cell cycle, however, it causes an increase in cell apoptosis. In turn, miR-331-3p accelerates the cell cycle and increases the apoptosis of Huh7.5 cells. Then the impact of the abovementioned miRNAs on HCV replication *in vitro* was assessed. For the first time it was shown that miR-106b-3p, miR-331-3p and miR-335-3p reduce HCV replication in Huh7.5 cells. Next, for these miRNAs putative target mRNAs were selected based on *in silico* analyzes. One of selection criterion was importance of those mRNAs for the regulation of HCV life cycle and/or development of liver damage in the course of CHC. For the first time direct interaction of miR-106b-3p with *STAT5B* and miR-335-3p with *PNPLA3* has been demonstrated. Binding of studied miRNAs within 3' UTRs of the abovementioned genes causes a decrease in their expression at the mRNA and protein level in Huh7.5 cells. However, reported decrease in HCV replication under the influence of miR-106b-3p and miR-335-3p does not occur directly by decreasing expression of *STAT5B* and *PNPLA3* by these molecules. On the contrary, silencing of *STAT5B* resulted in the increased HCV replication and infectivity. In contrast, *PNPLA3* silencing did not affect any of these processes. It was also shown that occurrence of SNP rs886057602 at the miR-335-3p binding site within the 3' UTR of *PNPLA3* attenuates miRNA-mediated repression of *PNPLA3* expression. Occurrence of SNP rs886057602 was not found in neither of studied CHC patients nor healthy donors.

The results obtained in the study indicate the significance of miR-106b-3p, miR-331-3p and miR-335-3p in the development and course of CHC. It was found that the expression analysis of the studied miRNAs in plasma is not useful for prognosis the course of CHC and predicting response to pegIFN-α and RBV treatment. It seems that miR-106b-3p, miR-331-3p and miR-335-3p through the modulatory effect on studied cell processes as well as negative regulation of HCV replication can affect the course of infection, the development of pathological lesions in the liver and the survival of the virus. Understanding the biological role of these miRNAs and identification the signaling pathways through which they affect HCV replication and hepatocyte function requires further study. Identification of miRNAs together with their target mRNAs may, in the long run, be useful for developing prognostic markers as well as new treatment approaches for CHC.