IDENTIFIES ELEVEN CIRCULATING miRNAs FOR DIAGNOSIS OF NASH AND FIBROSIS

The prevalence of NASH is rapidly growing and represents a major public health issue. NAFLD drives progressive fibrosis in a subset of patients ultimately leads to cirrhosis and hepatocellular carcinoma (HCC). Although there is no current pharmacological treatment for NASH, many new drug candidates are currently in phase 2 and phase 3 and new treatments should be available in the coming years.

Liver biopsy remains the only method for diagnosis of NASH and for scoring disease activity and stage of liver fibrosis. However, biopsy is invasive and cannot be used in the large number of patients that may be in need for pharmacotherapy. There is an urgent need for new and easily available non-invasive diagnostic methods for NASH.

Micro-RNAs (miRNAs) can play an important role in regulating gene expression in the extracellular space and body fluids, where they remain remarkably stable. Circulating miRNAs can potentially reflect the disease activity and stage and could provide an alternative method to assess NASH patients. However, the value of miRNAs as potential diagnostic or prognostic biomarkers for NASH is limited as they are not entirely specific, since miRNAs are released by necrotic or apoptotic cells and are not entirely specific to NASH. Therefore, careful selection of miRNAs is necessary.

Several reports have explored miRNA in circulation of control, simple steatosis and NASH patients, confirming promise of using miRNAs as potential diagnostic or prognostic biomarkers for NASH (see Alfonso MB et al., J. Clin. Med. 2016;5(3):30). These studies mainly measured plasma/serum levels of a limited number of miRNAs, which were selected based on previous reports of their association with liver diseases, cancer, or CVD.

Our aim was to perform an unbiased identification of miRNAs differentially expressed in serum samples from patients with NASH compared vs To-Be-Treated (TBT) vs NTBT samples from patients without indication for pharmacotherapy because of low disease activity and/or low fibrosis stage (Not-To-Be-Treated). We used two large independent cohorts of patients with scored liver biopsy and corresponding serum samples. Circulating levels of 2083 miRNA species were simultaneously measured and discriminating miRNAs were then confirmed by a classical RT-qPCR approach.

RESULTS: HTG-EdgeSeq-NGS

Volcano-plots obtained in GOLDEN-Diag and OBESE comparing fold change and p-value of each individual miRNA in TBT vs NTBT patients

Commonly over-expressed miRNAs (TBT vs NTBT) with fold change +1.3 in GOLDEN-Diag and OBESE

Volcano plots derived from comparison of serum levels at 2083 different miRNAs in TBT and NTBT are presented in figure 1. There were more over-expressed that under-expressed miRNAs when selection criteria were 35 times higher in expression in TBT vs NTBT in GOLDEN-Diag and 17 times in OBESE.

MiRNAs were considered differentially expressed if based on statistical significance (p<0.01) and fold change (1.3 in TBT vs NTBT). 23 were selected in GOLDEN-Diag and 16 were selected in OBESE.

After removing miRNAs with low expression levels in GOLDEN-Diag (<100 reads in both TBT and NTBT), cross-validation between the two cohorts gave significant (p<0.01) in the two cohorts. Differentially expressed miRNAs were sorted by fold change and/or statistical significance (p<0.01) for each individual miRNA in TBT vs NTBT patients.

CONCLUSION

This analysis of more than 500 NAFLD patients from two independent cohorts by NGS technology, allowed a non-biased selection of the most discriminating circulating miRNAs associated with NASH and liver fibrosis. From a total of 2083 miRNA levels in miRBase, 11 miRNAs were significantly over expressed in TBT vs NTBT in two independent cohorts of patients. In addition to miR34a and mir122, 9 new miRNAs were significantly associated with To-Be-Treated condition, NAS≥4 and F≥2.

RT-qPCR experiments confirmed that miR122, miR34a and miR122 were discriminating miRNAs in both cohorts. miR122 had high discriminating potentials in both cohorts and their serum levels significantly increased with NAS and fibrosis stage. miR34a had a high discriminating potential in both cohorts but there was no significant correlation with NAS and fibrosis stage in OBESE.

CONCLUSION

RT-qPCR experiments confirm a strong differential expression of both mir34a and mir122 in the two cohorts. In both cohorts, miR34a serum levels gradually increase with NAS and fibrosis.

In contrast mir122 levels increased with NAS in both cohorts but not with fibrosis in the GOLDEN-Diag cohort.