Non-alcoholic fatty liver disease (NAFLD) progression to non-alcoholic steatohepatitis (NASH) and NASH-related hepatocellular carcinoma (HCC) evolves following a differential activation of endoplasmic reticulum stress responses

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is notoriously heterogeneous in both its pathophysiology and clinical progression phenotypes. Endoplasmic reticulum stress and the coping response through the Unfolded Protein Response (UPR) have shown to play a significant role in NAFLD-related lipotoxicity. Nevertheless, the activation of the UPR in human and mouse NAFLD progression has not been described in detail. With the current changing of liver disease demographic away from viral etiology to metabolic causes due to more effective treatment, there is an important need to investigate the characteristics and behaviour of liver disease with metabolic etiology.

AIM

The main aim of this study was to gain a better understanding of the UPR activation in NAFLD progression using transcriptomic data of existing patient cohorts, gaining a better understanding of UPR activation patterns and its relation to the chronology of NAFLD progression to NASH and NASH HCC.

METHODS

Based on previous knowledge, six main regulators were identified as indicators of activation of different UPR-related pathways, namely: PERK/ATF4, IRE1A/XBP1 and ATF6. SIRT3 was considered the main regulator of the mitochondrial UPR. An exhaustive compilation of gene expression signatures included curated collections from the Molecular Signature Database (MSigDB) and was completed by using knockin and knockout data from published sources. RNA-sequencing data from tunicamycin-treated and ATF6-null HCC cell lines generated in our laboratory were used as bonafide control gene sets. *Mus musculus* gene symbols were replaced by their human orthologs. Gene set variation analysis (GSVA) and single sample Gene Set Enrichment Analysis (ssGSEA) were used to analyze the enrichment of the UPR signatures in human and mouse expression datasets. Associations between UPR enrichment data and histological data were analysed. Statistical analysis was performed using t-student for comparing means of normally distributed continuous variables and Chi-Square was used for comparing categorical variables.

RESULTS

From forty-seven preliminary signatures in both Homo sapiens and Mus Musculus, a final set of sixteen bona fide UPR signatures were derived: 2 for ATF4, 2 for ATF6, 3 for IRE1A, 7 for XBP1, 1 for PERK and 1 for SIRT3 signatures. Using GSVA and ssGSEA these signatures were applied to two NAFLD progression (n = 304 patients, NAFLD progression F0-4) and two NASH-Hepatocellular Carcinoma (NASH-HCC) (n = 180 samples, including Normal, Cirrhosis, NASH, NASH-HCC and peritumoural counterparts), human data sets. It was seen that there are differential expression patterns seen in the progression from healthy to NASH and NASH-HCC (Figure 1). Using hierarchical clustering the samples were sorted into 3 groups based on overall expression (Figure 2). These patterns were significally associated with the degree of fibrosis and NAS score.

CONCLUSIONS

- During progression from healthy livers to NASH and NASH associated cirrhosis and HCC there is a differential transcriptional regulation of the endoplasmic reticulum stress responses.
- The UPR effectors PERK, ATF4 and the mitochondrial stress response regulator Sirt3 show an up regulation during the development of NAFLD and NASH, while they become down regulated in cirrhosis.
- The IRE1 pathway and the ATF6 activity show some more heterogeneous expression patterns during NAFLD progression.
- The three arms of the UPR, namely PERK/ATF4, IRE1/XBP1, ATF6 showed activation in NASH HCC

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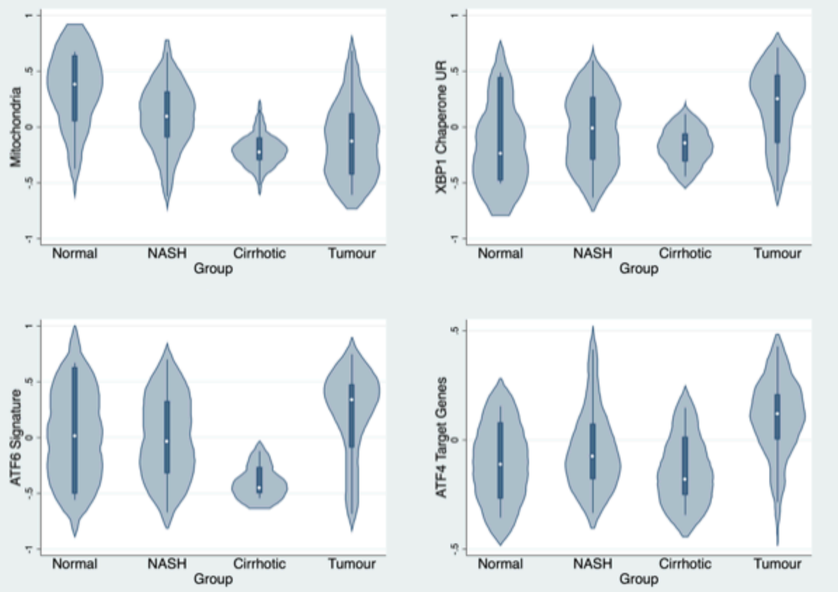

Figure 1. Violin-plots showing expression of signatures with respect to group

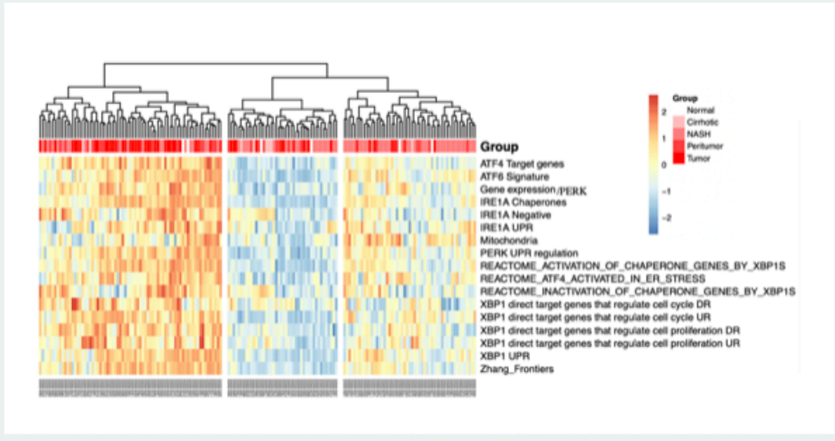

Figure 2. Heatmap showing expression of signatures with respect to group

Gene Signature	p-value comparison 'group' and gene signature	p-value comparison expression of signatures in NASH versus Tumour
ATF4 Target genes	0.0000	0.0019
ATF6 Signature	0.0002	0.0455
Gene expression/PERK	0.0000	0.0005
IRE1A Chaperones	0.0001	0.0062
IRE1A Negative	0.0354	0.0254
IRE1A UPR	0.0062	0.0538
Mitochondria	0.0001	0.0023
PERK UPR regulation	0.0244	0.1015
XBP1 Chaperon UR	0.0001	0.0042
ATF4 ER STRESS	0.1109	0.3630
XBP1 Chaperone DR	0.8171	0.6709
XBP1 DTG cell cycle DR	0.8171	0.0000
XBP1 DTG cell cycle UR	0.0014	0.0037
XBP1 DTG cell proliferatic	0.0000	0.0000
XBP1 DTG cell proliferatic	0.0000	0.0000
XBP1 UPR	0.0000	0.0024
Zhang_Frontiers	0.0000	0.0000

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