Comprehensive profiling of clock genes expression in hepatocellular carcinoma (HCC)

Introduction

• Disruption of the circadian clock modulating cellular endogenous 24-hour rhythms is associated with cancer risk, development and progression.

• Core clock proteins are recently emerging as novel therapeutic targets in cancer.

• The circadian clock mechanism controls the physiological homeostasis of the liver and plays a key role in hepatocarcinogenesis. Our group showed that clock regulators BMAL1 and CLOCK can promote proliferation of liver cancer cells by modulating the cell cycle checkpoint kinase Weel.1

• Here we further evaluated the molecular landscape of clock pathway alterations in HCC.

Methods

• A total of 780 HCC tested at Caris Life Sciences (Phoenix, AZ) with WTS (illumina Novaseq) and NextGen DNA sequencing (NextSeq, 5902 genes and Novaseq, WES) were analyzed.

• A clock gene Score (CS) was determined using expression of core clock genes 2 scores (positives of CLOCK, ARNTL, RORC, and negatives of repressors CRYY2, PER1/2, REVERRBα/b) stratified by quartiles (Q1 to Q4 - high).

• iCell was used to quantify cell infiltration in the tumor microenvironment (TME).

• X’ and Fisher-Exact tests were used for comparison and significance was determined as P-values adjusted for multiple testing (q ≤ 0.05).

• Gene expression profiles were analyzed for transcriptional signatures predictive of response to immunotherapy including the T cell costimulatory (TIS) and IFG score.

• Real world survival was obtained from insurance claims data and Kaplan-Meier estimates were calculated for comparison.

Results

Figure 1. Patients Characteristics and CS Distribution According to Sample Type.

- CS was higher in metastatic sites than primary tumors (median transcripts per million (TPM): Q1 81.05 ≤ P > 0.05).

- No significant differences in patient age and sex were observed between CS Q1 (lowest) and Q4 (highest) cohorts, although a trend towards a higher frequency of males was observed in Q4 (76% vs 68%; Q1 vs Q4, P = 0.07).

Figure 2. Tumor Molecular Characteristics According to CS.

- CS was positively associated with telomerase subunit TERT mutations (64% vs 52%; Q4 vs Q1, P = 0.04) and negatively correlated with FG3D copy number amplification (2% vs 6%; P = 0.04) and iWEST gene expression (median TPM 15 vs 28, q ≤ 0.05).

- Expression of immune related genes was lower in tumors with high CS, including ID01, CD86, PD-L1, LAG3, C306, TIM3, PD-1 and PD-L2 (fold change [FC]: 0.57-0.67 q ≤ 0.05).

Figure 3. Immune-related Markers.

- No dMMR/MSH-H tumors were observed in our series and there were no significant associations with tumor mutational burden and PD-L1 protein expression.

Figure 4. Immune-related Gene Expression According to CS.

- Expression of immune related genes was lower in tumors with high CS, including ID01, CD86, PD-L1, LAG3, C306, TIM3, PD-1 and PD-L2 (fold change [FC]: 0.57-0.67 q ≤ 0.05).

Conclusions

• This is the most extensive profiling study to investigate the expression of clock genes in HCC.

• Our data show that clock genes expression impacts patient survival and is associated with alterations in core related immune gene expression and TIS score which suggest a role in the modulation of anti-tumor immunity.

• These results support the clock pathway role as an oncogenic driver and its potential as a therapeutic target in HCC.