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

Francesca Battaglin[1], Yasmine Baca[2], Joanne Xiu[2], Shivani Soni[1], Sandra Algaze[1], Priya Jayachandran[1], Evanthia T. Roussos Torres[1], Shannon M. Mumenthaler[1,3], Jae Ho Lo[1], Pooja Mittal[1], Wu Zhang[1], Richard M. Goldberg[4], Benjamin A. Weinberg[5], Emil Lou[6], Anthony F. Shields[7], John L. Marshall[5], Sanjay Goel[8], Anthony El-Khoueiry[1], Steve A. Kay[9], Heinz-Josef Lenz[1]

1. Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, CA; 2. Caris Life Sciences, Phoenix, AZ; 3. Lawrence J. Ellison Institute for Transformative Medicine, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, CA; 4. West Virginia University Cancer Institute, Morgantown, WV; 5. Ruesch Center for The Cure of Gastrointestinal Cancers, Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC; 6. Division of Hematology, Oncology and Tranplantation, University of Minnesota, Minneapolis, MN; 7. Department of Oncology, Karmanos Cancer Institute, Wayne State University, Detroit, MI; 8. Rutgers Cancer Institute of New Jersey, New Brunswick, NJ; 9 Department of Neurology, Keck School of Medicine, University of Southern California, Los Angeles, CA.

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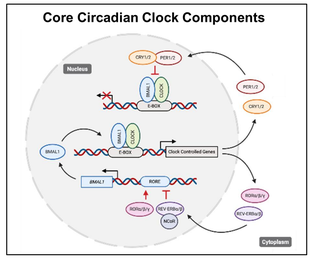
fbattagl@usc.edu

Introduction

- Disruption of the circadian clock modulating cellular endogenous 24-hour rhythms has been associated with cancer risk, development and progression.
- Core clock proteins are recently emerging as novel therapeutic targets in cancer.[1]
- 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 Wee1.[2]
- Here we further evaluated the molecular landscape of clock pathway alterations in HCC.



1. Battaglin et al. Oncogene 2021; 2. Meng et al. PNAS 2022.

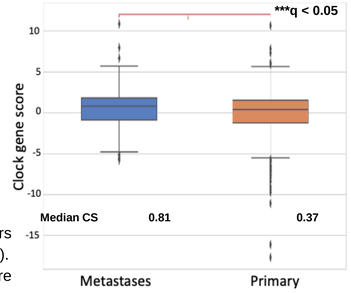
Methods

- A total of 780 HCC tested at Caris Life Sciences (Phoenix, AZ) with WTS (Illumina NovaSeq) and NextGen DNA sequencing (NextSeq, 592 Genes and NovaSEQ, WES) were analyzed.
- A clock gene Score (CS) was determined using expression of core clock genes Z scores (positives of *CLOCK*, *ARNTL*, *RORA/B/C* and negatives of repressors *CRY1/2*, *PER1/2/3*, *REVERBA/B*) stratified by quartiles (Q1 = low, Q4 = high).
- xCell was used to quantify cell infiltration in the tumor microenvironment (TME).
- X^2 and Fisher-Exact tests were used for comparison and significance was determined as *P*-values adjusted for multiple testing (*q*) of < 0.05.
- Gene expression profiles were analyzed for transcriptional signatures predictive of response to immunotherapy including the T cell inflamed score (TIS) and IFG score.
- Real world survival was obtained from insurance claims data and Kaplan-Meier estimates were calculated for comparison.

Results

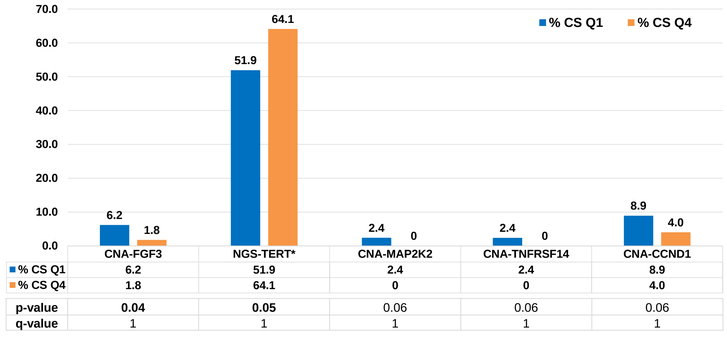
Figure 1. Patients Characteristics and CS Distribution According to Sample Type.

	CS Q1	CS Q4
Count (N)	195	195
Median Age [range]	67 [13 - >89]	66 [13 - >89]
Male	67.7%	75.9%
Female	32.3%	24.1%
Median TMB [range]	4.0 [0.0 - 43.0]	4.0 [0.0 - 13.0]

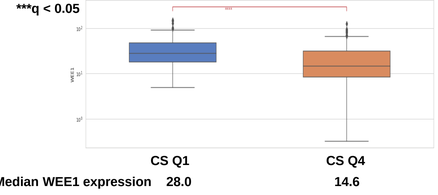


- CS was higher in metastatic sites than primary tumors (median transcripts per million [TPM]: 0.81 vs 0.37, *P* < 0.05).
- No significant differences in patient age and sex were observed between CS Q1 (lowest) and Q4 (highest) cohorts, although a trend towards a higher frequency of males was observed in Q4 (76% vs 68%, Q4 vs Q1, *P* = 0.07).

Figure 2. Tumor Molecular Characteristics According to CS.

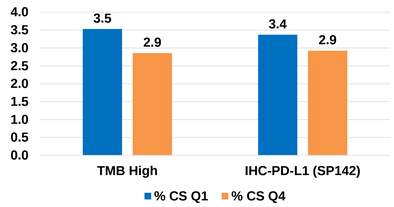


	CNA-FGF3	NGS-TERT*	CNA-MAP2K2	CNA-TNFRSF14	CNA-CCND1
% CS Q1	6.2	51.9	2.4	2.4	8.9
% CS Q4	1.8	64.1	0	0	4.0
p-value	0.04	0.05	0.06	0.06	0.06
q-value	1	1	1	1	1



- CS was positively associated with telomerase subunit TERT mutations (64% vs 52%, Q4 vs Q1, *P* = 0.04) and negatively correlated with *FGF3* copy number amplification (2% vs 6%, *P* = 0.04) and *WEE1* gene expression (median TPM: 15 vs 28, *q* < 0.05).

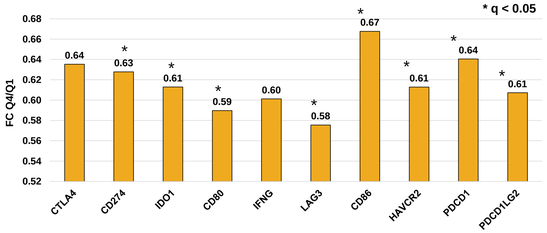
Figure 3. Immune-related Markers.



PDL-1 cutoff 2+ 5%

- No dMMR/MSI-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.

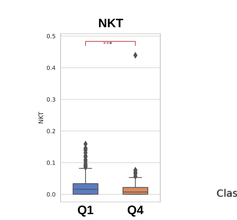


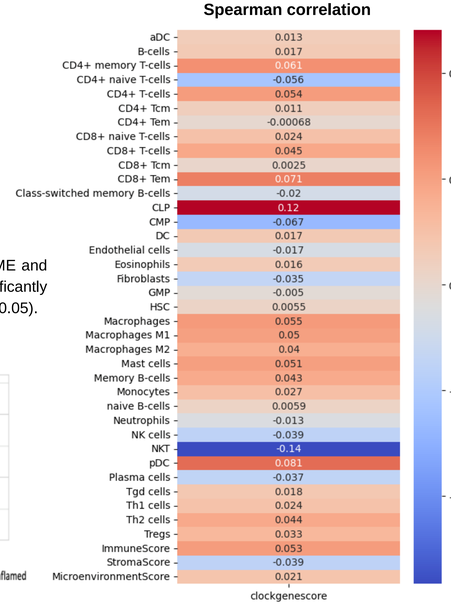
- Expression of immune related genes was lower in tumors with high CS, including *IDO1*, *CD80*, *PD-L1*, *LAG3*, *CD86*, *TIM3*, *PD-1* and *PD-L2* (fold change [FC]: 0.57-0.67 *q* < 0.05).

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 immune-related gene expression and TIS score which suggest a role in the modulation of anti-tumor immunity.**
- **These results support the clock pathway role as a oncogenic driver and its potential as a therapeutic target in HCC.**

Figure 5. Immune Cell Infiltration and TIS Score According to CS.





- NK cell infiltration in the TME and the TIS score were significantly lower in CS-high HCC (*q* < 0.05).

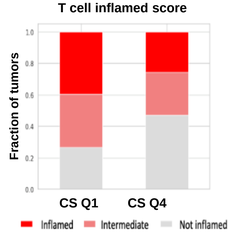
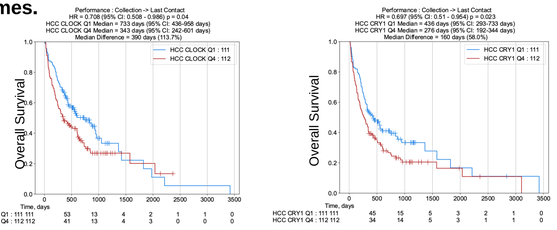


Figure 6. Association between Clock Gene Expression and Patient Outcomes.



- Lower *CLOCK* and *CRY1* tumor mRNA expression were associated with longer OS (Q1 vs Q4: *CLOCK* HR 0.71, 95%CI [0.51-0.98], *P* = 0.04 and *CRY1* HR 0.70 [0.51-0.95], *P* = 0.02, respectively).