Comprehensive profiling of clock genes expression in colorectal cancer (CRC)

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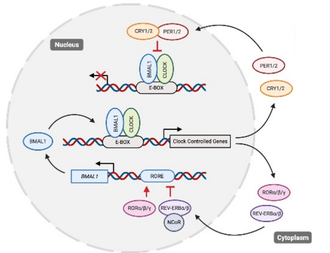
Introduction

- 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]
- We previously showed that polymorphisms in clock genes were associated with anti-VEGF treatment outcome in metastatic CRC.[2,3]
- Here we further evaluated the molecular landscape of clock pathway alterations in CRC.

Core Circadian Clock Components



Methods

- A total of 7,591 CRC 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).
- Consensus molecular subtypes (CMS) were assessed by RNAseq.
- 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.
- Real world survival was obtained from insurance claims data and Kaplan-Meier estimates were calculated for comparison.

1. Battaglin et al. Oncogene 2021; 2. Battaglin et al. ASCO Annual Meeting 2018; 3. Battaglin et al. ESMO Congress 2020.

Results

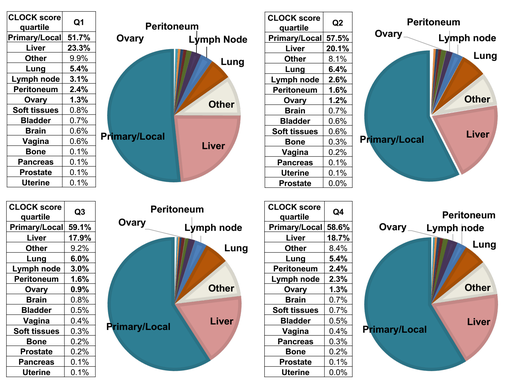
Figure 1. CS Distribution According to Sample Type, Primary Tumor Location and CMS.

CLOCK score quartile	Q1
Primary/Local	51.7%
Liver	23.3%
Other	9.9%
Lung	5.4%
Lymph node	3.1%
Peritoneum	2.4%
Ovary	1.3%
Soft tissues	0.8%
Bladder	0.7%
Brain	0.6%
Vagina	0.6%
Bone	0.1%
Pancreas	0.1%
Prostate	0.1%
Uterine	0.1%

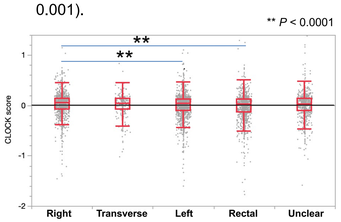
CLOCK score quartile	Q2
Primary/Local	57.5%
Liver	20.1%
Other	8.1%
Lung	6.4%
Lymph node	2.6%
Peritoneum	1.6%
Ovary	1.2%
Brain	0.7%
Bladder	0.6%
Soft tissues	0.6%
Bone	0.3%
Vagina	0.2%
Pancreas	0.1%
Uterine	0.1%
Prostate	0.0%

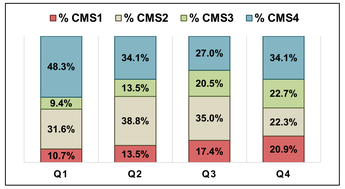
CLOCK score quartile	Q3
Primary/Local	59.1%
Liver	17.9%
Other	9.2%
Lung	6.0%
Lymph node	3.0%
Peritoneum	1.6%
Ovary	0.9%
Brain	0.8%
Bladder	0.5%
Vagina	0.4%
Soft tissues	0.3%
Bone	0.2%
Prostate	0.2%
Pancreas	0.1%
Uterine	0.1%

CLOCK score quartile	Q4
Primary/Local	58.6%
Liver	18.7%
Other	8.4%
Lung	5.4%
Peritoneum	2.4%
Lymph node	2.3%
Ovary	1.3%
Brain	0.7%
Soft tissues	0.7%
Bladder	0.5%
Vagina	0.4%
Pancreas	0.3%
Bone	0.2%
Prostate	0.1%
Uterine	0.0%



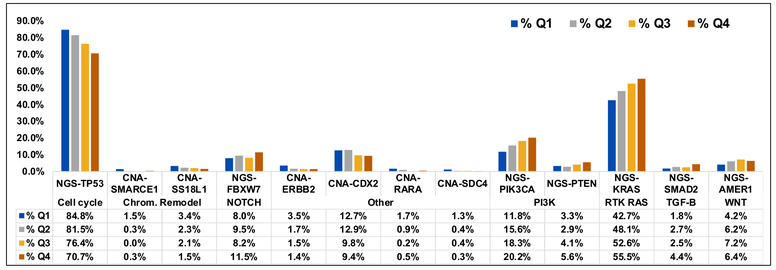
- CS was higher in primary tumors than metastases and in right- than left-sided CRC (*P* < 0.001).
- Liver metastases were associated with lower CS (23% Q1 vs 19% Q4, *P* < 0.001).





- CS was positively associated with CMS1 and 3 (21 vs 11% and 23 vs 9%, respectively, Q4 vs Q1) and negatively correlated with CMS2 and 4 (22 vs 32% and 34 vs 48%) (all *P* < 0.001).
- These associations were confirmed in mismatch repair proficient (pMMR) tumors.

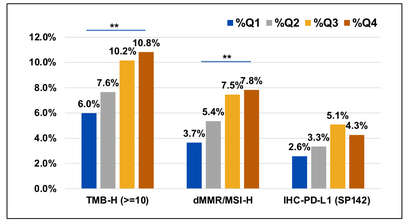
Figure 2. Tumor Molecular Characteristics According to CS (MSS Cohort).



	NGS-TP53	CNA-SMARCE1	CNA-SS18L1	NGS-FBXW7	CNA-ERBB2	CNA-CDX2	CNA-RARA	CNA-SDC4	NGS-PIK3CA	NGS-PTEN	NGS-KRAS	NGS-SMAD2	NGS-AMER1
	Cell cycle	Chrom. Remodel		NOTCH	Other				PI3K		RTK RAS	TGF-B	WNT
% Q1	84.8%	1.5%	3.4%	8.0%	3.5%	12.7%	1.7%	1.3%	11.8%	3.3%	42.7%	1.8%	4.2%
% Q2	81.5%	0.3%	2.3%	9.5%	1.7%	12.9%	0.9%	0.4%	15.6%	2.9%	48.1%	2.7%	6.2%
% Q3	76.4%	0.0%	2.1%	8.2%	1.5%	9.8%	0.2%	0.4%	18.3%	4.1%	52.6%	2.5%	7.2%
% Q4	70.7%	0.3%	1.5%	11.5%	1.4%	9.4%	0.5%	0.3%	20.2%	5.6%	55.5%	4.4%	6.4%

- High CS was associated with alterations of genes in WNT signaling, RAS, PI3K, TGF-β, and NOTCH pathways, while negatively associated with *TP53* mutations, *HER2* expression and *CDX2* copy numbers, confirmed in pMMR (all *q* < 0.05).

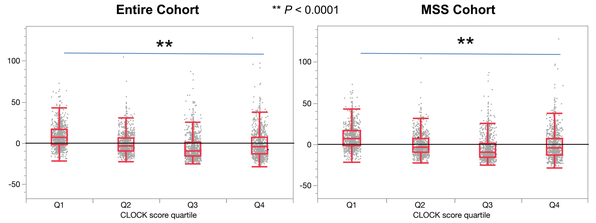
Figure 3. Immune-Related Markers.



* *q* < 0.0001; PDL-1 cutoff 2+ 5%

- Overall, TMB-H and dMMR/MSI-H were positively associated with CS (11 vs 6% and 8 vs 4%, Q4 vs Q1, *q* < 0.0001) and PD-L1 showed a similar trend (*P* < 0.01, *q* = 0.06).
- The association with TMB-H was not significant in pMMR.

Figure 4. Angiogenesis Pathway Z Score Analysis.

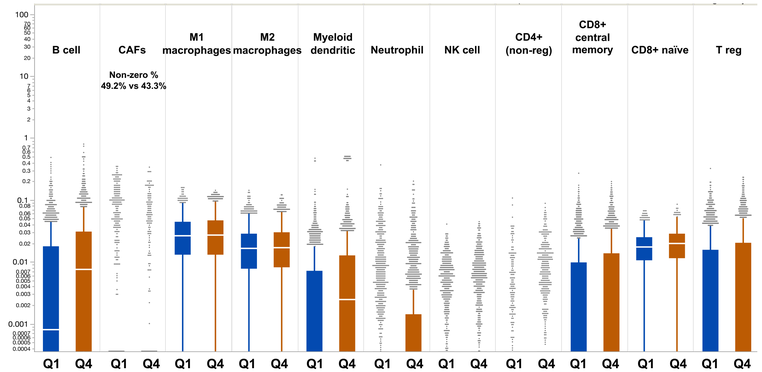


- CS negatively correlated with the angiogenesis pathway signature (Q1 vs Q4 Z score: 6.6 vs -4.6, *P* < 0.001).

Conclusions

- **This is the most extensive profiling study to investigate the expression of clock genes in CRC.**
- **Our data show that clock genes expression is strongly associated with distinct molecular features, immune cell infiltration, angiogenesis pathway enrichment and patient outcomes.**
- **These findings support the clock pathway as a therapeutic target in CRC, with a major role in CRC biology and TME modulation.**

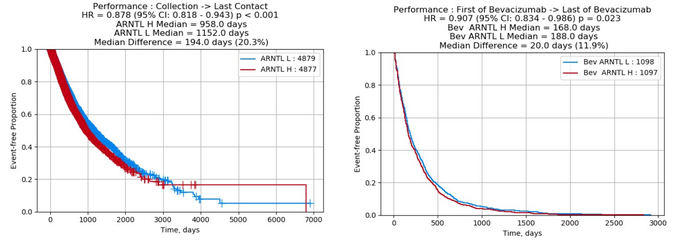
Figure 5. Immune Cell Infiltration According to CS (MSS Cohort).



- B cells, M1 and M2 macrophages, neutrophils, NK, Tregs, CD4+ and CD8+ T cells (central memory and naïve), and myeloid dendritic cells were more abundant in the TME of tumors with high CS while cancer associated fibroblasts (CAFs) were lower, regardless of MMR status (all *p* < 0.001).
[Table shows median infiltrate]

Immune cell (xCell)	Q1 (median infiltrate)	Q4 (median infiltrate)
B cel	0.000796248	0.007622656
CAFs	-	-
M1 Macrophages	0.0262837	0.027202871
M2 Macrophages	0.016524575	0.016962422
Myeloid dendritic cells	2.16E-18	0.002438705
Neutrophil	2.05E-19	4.39E-19
NK cells	5.81E-19	8.49E-19
CD4+T (non-reg)	1.60E-20	1.29E-19
CD8+ T (central memory)	2.71E-18	1.09E-17
CD8+ T (naïve)	0.017459106	0.019593494
Tregs	6.26E-18	1.94E-17

Figure 6. Association between *ARNTL* Expression and Patient Outcomes.



- *ARNTL* (*BMAL1*) tumor expression below median was associated with better OS (overall: HR 0.88, 95% CI [0.82-0.94]; pMMR: HR 0.88 [0.81-0.94]) and longer time on treatment of bevacizumab (overall: HR 0.91 [0.83-0.99]; pMMR: HR 0.91 [0.83-0.99]).