Clock Genes in Breast Cancer

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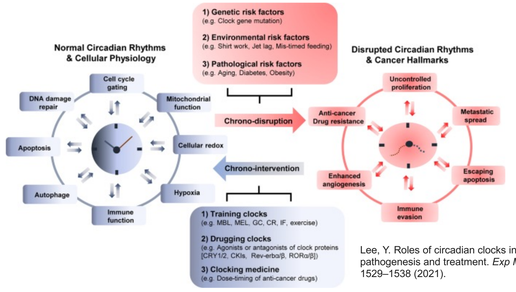
Background

- Disruption of circadian processes has been linked to cancer initiation, progression, metastasis, resistance, and mortality.
- Clock proteins are an emerging target for therapy in breast cancer.



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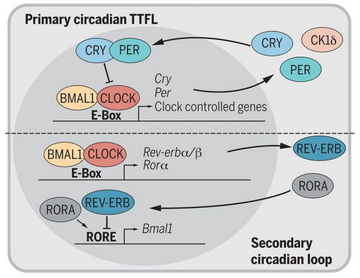
Clock Mechanism



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- Circadian rhythms are controlled by a network of transcription/translation feedback loops primarily driven by BMAL and CLOCK and the transcriptional repressors period (PER1-3) and cryptochrome (CRY1-2).
- We investigated the molecular and clinical associations of clock genes in breast cancer.

Methods

- A total of 9563 breast tumors underwent molecular profiling (Caris Life Sciences).
- Analyses included next- generation sequencing of DNA (592 genes, NextSeq, WES, NovaSEQ) and RNA (NovaSeq).
- Clock gene Score (CS) was determined using expression of core clock pathway gene Z scores (positives of BMAL, CLOCK and negatives of PER1/2 and CRY1/2) and then stratified into quartiles.
- xCell was used to quantify the immune cell infiltration in the tumor microenvironment (TME).
- ER/PR was tested by IHC and HER2 was tested by either IHC or CISH.
- Significance was determined as *P* values adjusted for multiple comparison (*Q*) of < .05. Real-world overall survival information was obtained from insurance claims data and was calculated from tissue collection to last contact; comparison done by Kaplan-Meier test.

Results

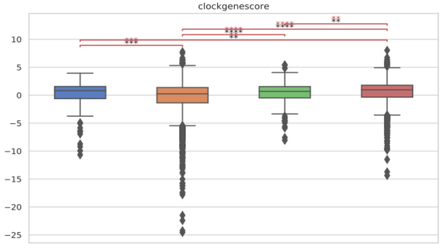
1. Clock gene score

GENE	Positive feedback	Negative Feedback	
CLOCK	X		Clock score
BMAL1 (ARNTL)	X		
PER 1		X	
PER 2		X	
CRY 1		X	
CRY 2		X	

2. Distribution of breast molecular subtypes

	TNBC	HR+/HER2-	HR+/HER2+	HR-/HER2+	Other	Total
Count (N)	2484	5140	397	291	1251	**9563**

3. Clock gene score in breast molecular subtypes



4. Tumor microenvironment (xcell) for A. TNBC and B. HR+/HER2-

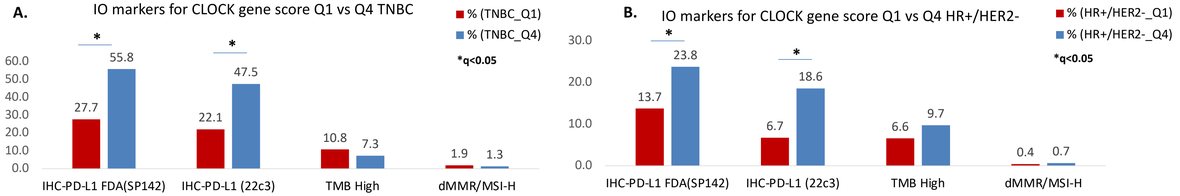
A.

Immune cell (xCell)	TNBC_Q1 (Median)	TNBC_Q4 (Median)	q-value
Myeloid dendritic cell activated	0.253889	0.328335	****<0.001**
B cell	6.33E-19	0.002386	****<0.001**
T cell CD4+ memory	0.022207	0.045155	****<0.001**
T cell CD4+ naive	0.002161	0.002861	N.S.
T cell CD4+ (non-regulatory)	6.15E-20	3.43E-19	****<0.001**
T cell CD4+ central memory	0.011173	3.68E-18	****<0.001**
T cell CD4+ effector memory	2.82E-18	1.86E-18	**0.017**
T cell CD8+ naive	0.021326	0.027247	****<0.001**
T cell CD8+	0.018134	0.02823	****<0.001**
T cell CD8+ central memory	4.32E-18	0.003646	****<0.001**
T cell CD8+ effector memory	4.61E-19	7.96E-19	N.S.
Class-switched memory B cell	0.005284	0.016363	****<0.001**
Common lymphoid progenitor	0.603307	0.739722	****<0.001**
Common myeloid progenitor	0 (38%)	0 (35%)	N.S.
Myeloid dendritic cell	0.00234	0.008431	****<0.001**
Endothelial cell	0.070049	0.055703	****<0.001**
Eosinophil	3.67E-19	1.28E-20	**0.002**
Cancer associated fibroblast	1.09E-17	2.83E-18	****<0.001**
Granulocyte-monocyte progenitor	3.27E-18	2.49E-18	N.S.
Hematopoietic stem cell	0.250199	0.202434	****<0.001**
Macrophage	0.050102	0.061457	****<0.001**
Macrophage M1	0.033201	0.041244	****<0.001**
Macrophage M2	0.033064	0.031188	N.S.
Mast cell	0.013666	0.015796	**0.003**
B cell memory	9.28E-19	1.81E-18	****<0.001**
Monocyte	7.05E-18	0.008106	****<0.001**
B cell naive	1.27E-19	7.33E-19	****<0.001**
Neutrophil	9.94E-20	1.50E-19	N.S.
NK cell	5.35E-20	6.89E-19	**0.028**
T cell NK	0.052283	0.040422	****<0.001**

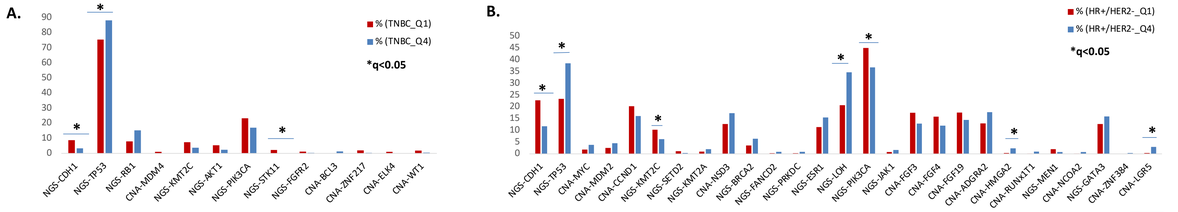
B.

Immune cell (xCell)	HR+/HER2- Q1 (Median)	HR+/HER2- Q4 (Median)	q-value
Myeloid dendritic cell activated	0.211262	0.238678	****<0.001**
B cell	3.81E-19	2.38E-18	****<0.001**
T cell CD4+ memory	0.017613	0.034434	****<0.001**
T cell CD4+ naive	0.008363	0.005215	N.S.
T cell CD4+ (non-regulatory)	1.57E-19	1.85E-19	N.S.
T cell CD4+ central memory	0.024243	0.001768	****<0.001**
T cell CD4+ effector memory	2.42E-18	2.17E-18	N.S.
T cell CD8+ naive	0.012221	0.018806	****<0.001**
T cell CD8+	0.011215	0.012161	N.S.
T cell CD8+ central memory	2.00E-18	2.85E-18	N.S.
T cell CD8+ effector memory	2.98E-19	7.29E-19	N.S.
Class-switched memory B cell	0.011206	0.016455	****<0.001**
Common lymphoid progenitor	0.59754	0.734689	****<0.001**
Common myeloid progenitor	0 (48%)	0 (40%)	**0.002**
Myeloid dendritic cell	0.000397	0.000618	N.S.
Endothelial cell	0.067142	0.051097	****<0.001**
Eosinophil	3.14E-19	1.85E-19	**0.036**
Cancer associated fibroblast	0.012825	8.44E-18	****<0.001**
Granulocyte-monocyte progenitor	3.27E-18	2.91E-18	N.S.
Hematopoietic stem cell	0.289781	0.233997	****<0.001**
Macrophage	0.0413	0.051587	****<0.001**
Macrophage M1	0.019178	0.024319	****<0.001**
Macrophage M2	0.033453	0.033033	N.S.
Mast cell	0.024383	0.024552	N.S.
B cell memory	8.60E-19	9.39E-19	N.S.
Monocyte	3.55E-18	3.59E-18	N.S.
B cell naive	2.66E-19	5.17E-19	**0.002**
Neutrophil	8.20E-20	7.19E-20	N.S.
NK cell	6.83E-19	1.16E-18	N.S.
T cell NK	0.078589	0.072115	**0.001**

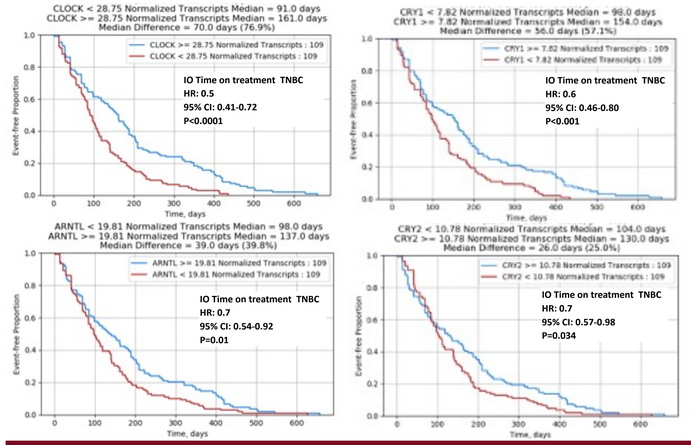
5. Markers of response to IO therapy for A. TNBC and B. HR+/HER2-



6. Significant and trending alterations associated with CS in A. TNBC and B. HR+/HER2-



7. Significant KM curves for Clock gene expression



Conclusions

- TNBC had the highest median CS while HR+/HER2- had the lost CS (0.96 vs 0.26 median TPM q<0.001).
- For IO related markers, PDL1 was associated with higher CS in both TNBC (56% Q4 vs 28% Q1, q<0.05) and in HR+/HER2- (24% Q4 vs 14% Q1, q<0.05).
- For the TME in TNBC, CD8+ T cells, B cells, monocytes and NK cells are positively associated with CS, whereas CD4+ central memory and CD4+ effector memory T cells, eosinophils, and endothelial cells are associated with lower CS (Q1 vs Q4 all q<0.05).
- In TNBC, TP53 mutations are associated with higher CS (88% Q4 vs 75% Q1), while CDH1 and STK11 mutations are associated with lower CS (3.4% Q4 vs 8.7% Q1 and 0.1% Q4 vs 2.2% Q1).
- For the TME in HR+/HER2- tumors, there is a decrease in CD4+ central memory cells as well as common myeloid progenitor cells in high CS tumors. There is also a decrease in endothelial cells and eosinophils in high CS tumors. Activated myeloid dendritic cells, B cells, CD4+ memory T cells, CD8+ naïve T cells, M1 macrophages and Tregs all have higher abundance in high CS tumors.
- In HR+/HER2- tumors, TP53 mutations, HMGA2 and LGR5 amplifications and LOH (WES) are all associated with higher CS (39% Q4 vs 23% Q1, 2% Q4 vs 0.4% Q1, 3% Q4 vs 0.4% Q1 and 35% Q4 vs 21% Q1). CDH1, KMT2C and PIK3CA mutations are associated with lower CS (12% Q4 vs 23% Q1, 6% Q4 vs 10% Q1 and 37% Q4 vs 45% Q1) (all q<0.05).
- Expression of Timeless (HR:0.7, CI: 0.65-0.77) and CLOCK (HR: 0.8, CI: 0.72-0.86) below median are associated with longer OS, while expression of CRY2 (HR: 1.4, CI: 1.3-1.6); PER2/3 (HR: 1.1, CI:1.0-1.2; HR:1.3, CI:1.2-1.4) above median are associated with longer OS. In TNBC, IO therapy from TOT survival is significantly prolonged with higher expression of CLOCK (HR: 0.5, CI: 0.41-0.72), TIMELESS (HR: 0.7 CI: 0.53-0.91), ARNTL (HR: 0.7 CI:0.54-0.92) and CRY1/2 (HR: 0.6, CI: 0.46-0.80; HR: 0.75 CI: 0.57-0.98) but not PER1/2.
- Clock genes are novel predictive and prognostic molecular markers and emerging targets for the development of new treatments in breast cancer.

References

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