Circadian Control of Xenobiotic Carcinogenesis and Anticancer Drug Pharmacology

Francis Lévi

Warwick Medical School

Rythmes Biologiques et Cancers

Paul Brousse hospital, Villejuif (France)
Circadian Control of Xenobiotic Carcinogenesis and Anticancer Drug Pharmacology

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Cancer Chronotherapy Unit
Cancer Research Unit

Rythmes Biologiques et Cancers

Paul Brousse hospital, Villejuif (France)
On the basis of “limited evidence in humans for the carcinogenicity of shift-work that involves nightwork”, and “sufficient evidence in experimental animals for the carcinogenicity of light during the daily dark period (biological night)”, the Working Group concluded that “shift-work that involves circadian disruption is probably carcinogenic to humans” (Group 2A).

Circadian Control of Cancerogenesis & Cancer Chronopharmacology

Systems Cancer Chronotherapeutics

The ten hallmarks of cancer

- Sustaining proliferative signaling
- Evading growth suppressors
- Deregulating cellular energetics
- Avoiding immune destruction
- Resisting cell death
- Enabling replicative immortality
- Genome instability & mutation
- Inducing angiogenesis
- Activating invasion & metastasis
- Cancer needs ≥ 6

Hanahan and Weinberg, Cell 2011

Cancer
Cancer cells
Leukocytes
Dendritic cellls
Blood vessels
The Circadian Timing System

- Clock genes
- Suprachiasmatic nuclei
- Sleep/wakefulness
- Rest/activity
- Environment
  - Day/night
  - Social
  - Familial
- Meal timing
  - Cortisol
  - Melatonin
- Temperature
- Feeding pattern
- Heart rate

Lévi et al. Annu Rev Pharm Toxicol 2010
Lévi & Okyar EODD 2011
Circadian Control of Cancerogenesis & Cancer Chronopharmacology

The Circadian Timing System

Environment
- Day/night
- Social
- Familial
- Meal timing

Suprachiasmatic nuclei
- Sleep/wakefulness

Rest-activity

Cortisol, melatonin, Temperature, Feeding pattern, Heart rate

Circadian clocks in peripheral organs

Circadian biomarkers

Rhythm monitoring in cancer patients

Chronotherapeutics
- Cell cycle, apoptosis, and DNA repair
- Drug metabolism and detoxification
- Angiogenesis

Lévi et al. Annu Rev Pharm Toxicol 2010
Lévi & Okyar EOODD 2011
Molecular clock

Clock gene transcription rhythms in human oral mucosa

Lévi et al. Annu Rev Pharm Toxicol 2010

Molecular clock

Clock-Controlled genes

- Drug metabolism and detoxification
  Cyp3a, Ces1-3, UGT1A1, GST-π, Upa, Dpyd,…

- Drug transport
  Abcb1a/b, Abcc2, Abcg2,…

- Drug targets
  TS, Top1, Top2, mTOR,…

- Cell cycle, apoptosis, repair
  Wee1, P21, P53, c-Myc, Bcl-2, Bax, Mdm2, cyclin D, Tip60,…

Lévi et al. Annu Rev Pharm Toxicol 2010
Circadian Control of Cancerogenesis & Cancer Chronopharmacology

Lévi & Schibler, Annu Rev Pharm Toxicol 2007
Lévi et al. Annu Rev Pharm Toxicol 2010
Circadian Control of Cancerogenesis & Cancer Chronopharmacology

- 5-Fluorouracil (5-FU)
- Irinotecan
- Oxaliplatin

Lévi & Schibler, Annu Rev Pharm Toxicol 2007
Lévi et al. Annu Rev Pharm Toxicol 2010
Circadian Control of Cancerogenesis & Cancer Chronopharmacology

Chronotolerance
40 anticancer drugs
Chronoeficacy
28 anticancer drugs

Lévi & Schibler, Annu Rev Pharm Toxicol 2007
Lévi et al. Annu Rev Pharm Toxicol 2010
**Circadian Timing System ↔ Cancer Progression at Whole Organism level**

<table>
<thead>
<tr>
<th>CTS disruption</th>
<th>Experimental cancer progression</th>
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<tbody>
<tr>
<td>• Suprachiasmatic ablation</td>
<td>Two- to three-fold acceleration</td>
</tr>
<tr>
<td>• Chronic jet lag</td>
<td></td>
</tr>
<tr>
<td>• ClockΔ19 mutation</td>
<td></td>
</tr>
</tbody>
</table>

| CTS re-inforcement                 |                                 |
|------------------------------------|                                 |
| • Meal Timing                      | Reduced by half to two-third     |

## Circadian Timing System ↔ Cancer Progression at Whole Organism level

### CTS disruption
- Suprachiasmatic ablation
- Chronic jet lag
- ClockΔ19 mutation

### Experimental cancer progression
- Two- to three-fold acceleration

### CTS re-inforcement
- Reduced by half to two-third

**Liver and tumor circadian transcriptome reprogramming**

Wu et al. Life Sci 2004; Li et al. Cancer Res 2010*
CTS disruption on liver cancerogenesis?

- Cancer promotion

Rat DEN model
CJL Mouse DEN model
CTS disruption on liver cancerogenesis?

- Cancer Initiation
- Cancer promotion

Rat DEN model

CJL Mouse DEN model
CTS disruption on liver cancerogenesis?

- Cancer Initiation
- Cancer promotion

**Rat DEN model**

**CJL Mouse DEN model**

**c-Myc upregulation**

*Fu et al. Cell 2002*

*Filipski et al. J Natl Cancer Inst 2005*
The DEN experimental cancer model

DiethylNitrosamine

Cyp2A5
Cyp2E1

α-hydroxyNitrosamine.

DNA adducts
Circadian Control of Cancerogenesis & Cancer Chronopharmacology

The DEN experimental cancer model

DiéthylNitrosamine

Cyp2A5
Cyp2E1
α-hydroxylNitrosamine.

Ethyl diazonium

DNA adducts

NDEA

CH₃CH₂

CH₃CH₂

CH₃CH₂

CH₃CH₂

CH₃CH₂

CH₃CH₂

NH²

N² + OH⁻

N = O + CH₃CHO

N = O

0₂, NADPH

TLR?

MyD88

NF-κB

Kupffer cell (macrophage)

Inflammatory tumour microenvironment

Repression of IL-6 production

Stimulation of IL-6 production

Hepatocellular carcinoma

Inflammation, cell proliferation

Liver cancer

Carcinogen (DEN)

Tissue damage

Oestrogen

Oestrogen
### Study I

<table>
<thead>
<tr>
<th></th>
<th>Study I</th>
<th>Study II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wt</td>
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</tr>
<tr>
<td></td>
<td>Per$_{2}^{m/m}$</td>
<td>Cry$_{1}^{-/-}$</td>
</tr>
<tr>
<td>Controls</td>
<td>2*</td>
<td>2</td>
</tr>
<tr>
<td>DEN**</td>
<td>13</td>
<td>16</td>
</tr>
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</table>

*N of mice

**DEN (5 d a week over 50 d at ZT11) = 402 mg/kg
# Circadian Control of Cancerogenesis & Cancer Chronopharmacology

## Study I
- Wt
- \( \text{Per}_2^{m/m} \)

## Study II
- Wt
- \( \text{Cry}_1^{-/-} \)-\( \text{Cry}_2^{-/-} \)

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- **N of mice**
- **DEN** (5 d a week over 50 d at ZT11) = 402 mg/kg

4 months

**Hepatocarcinoma**

**Cholangiocarcinoma**

*Mteyrek et al. submitted*
Circadian Control of Cancerogenesis & Cancer Chronopharmacology

**Per2\textsuperscript{m/m}**

Kruskal–Wallis ANOVA

\[ P = 0.01 \]

N of mice with >2 nodules

\textit{Wt} : 2/13 (15 %) ; \textit{Per2}\textsuperscript{m/m} : 8/10 (80 %)

\textit{Exact Fisher, p= 0.003}

\textit{Mteyrek et al. submitted}
**Circadian Control of Cancerogenesis & Cancer Chronopharmacology**

**Per2<sup>m/m</sup>**

- **Kruskall –Wallis ANOVA**
  - *P* = 0.01

<table>
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<th>Number of mice</th>
<th>Number of tumor nodules / liver</th>
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<tr>
<td>WT mice</td>
<td>2 1 2 4 6 7 8 9 10</td>
</tr>
<tr>
<td>Per2&lt;sup&gt;m/m&lt;/sup&gt; mice</td>
<td>0 1 2 3 4 5 6 7 8 9 10</td>
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- **N of mice with >2 nodules**
  - **Wt**: 2/13 (15 %)
  - **Per2<sup>m/m</sup>:** 8/10 (80 %)

*Exact Fisher, p= 0.003*

**Cry1<sup>−/−</sup>-Cry2<sup>−/−</sup>**

- **Kruskall –Wallis ANOVA**
  - *P* = 0.01

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<td>WT mice</td>
<td>0 1 2 3 4 5 6 7 8 9 10 11</td>
</tr>
<tr>
<td>Cry dKO mice</td>
<td>0 1 2 3 4 5 6 7 8 9 10 11 25</td>
</tr>
</tbody>
</table>

- **N of mice with >2 nodules**
  - **Wt**: 2/14 (14 %)
  - **Cry dKO**: 9/11 (82 %)

*Exact Fisher, p= 0.01*

**Mteyrek et al. submitted**
Early response of circadian gene expressions to DEN

Cancer Initiation

24 h after daily DEN at ZT11 for 3 wks

* 24-h rhythm (p<0.05)

Mteyrek et al. Submitted
Early response of circadian gene expressions to DEN

**Cancer Initiation**

*24 h after daily DEN at ZT11 for 3 wks*

* 24-h rhythm (p<0.05)

**Cell Cycle**

Mteyrek et al. Submitted
Early response of circadian gene expressions to DEN

Cancer Initiation

24 h after daily DEN at ZT11 for 3 wks

* 24-h rhythm (p<0.05)

Cell Cycle

Clock

Mteyrek et al. Submitted
Cancer Initiation

24 h after daily DEN at ZT11 for 3 wks

* 24-h rhythm (p<0.05)

Mteyrek et al. Submitted
Early response of circadian gene expressions to DEN

Cancer Initiation

24 h after daily DEN at ZT11 for 3 wks

* 24-h rhythm (p<0.05)

Clock dampening

Rhythm suppression

C-Myc derepression

Mteyrek et al. Submitted
Early response of circadian gene or protein expressions to DEN

Cancer Initiation

24 h after daily DEN at ZT11 for 3 wks

* 24-h rhythm (p<0.05)

Genomic Instability

- Bcl2
- P53
- ATM

Inflammation

- IL-6
- TNF-α
Early response of circadian gene or protein expressions to DEN

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24 h after daily DEN at ZT11 for 3 wks

* 24-h rhythm (p<0.05)
Circadian Control of Cancerogenesis & Cancer Chronopharmacology

Early response of circadian gene or protein expressions to DEN

Cancer Initiation

24 h after daily DEN at ZT11 for 3 wks

* 24-h rhythm (p<0.05)

Liver cytokines concentrations (pg/mg of proteins)

Genomic Instability

Bcl2
P53
ATM

Inflammation

II-6
TNF-α

**24 h after daily DEN at ZT11 for 3 wks**

* 24-h rhythm (p<0.05)
Circadian Control of Cancerogenesis & Cancer Chronopharmacology

**Early response of circadian gene or protein expressions to DEN**

* 24-h rhythm (p<0.05)

**Relative abundance**

- Liver cytokines concentrations (pg/mg of proteins)

**Genomic Instability**
- Bcl2
- P53
- ATM

**Cancer Initiation**

24 h after daily DEN at ZT11 for 3 wks

**Rhythm suppression**

**Inflammation**

↑inflammation
Circadian Control of Cancerogenesis & Cancer Chronopharmacology

Systems Cancer Chronotherapeutics

Circadian Clock On Cancer Hallmarks

Fu et al. Cell 2002
Filipski et al. JNCI 2005, Mut Res 2009
Mteyrek et al. EBRS 2013, submitted

After Hanahan and Weinberg, Cell 2011
Circadian Control of Cancerogenesis & Cancer Chronopharmacology

Systems Cancer Chronotherapeutics

After Hanahan and Weinberg, Cell 2011


c-Myc derepression

Circadian Clock On Cancer Hallmarks

P53 repression

Fu et al. Cell 2002
Filipski et al. JNCI 2005, Mut Res 2009
Mteyrek et al. EBRS 2013, submitted
Host circadian disruption speeds up cancer
Host circadian reinforcement inhibits cancer progression
Host circadian disruption speeds up cancer
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Host circadian disruption speeds up cancer
Host circadian reinforcement inhibits cancer progression

Host clocks control cancer
In vitro cancer chronopharmacology

• Coupling of circadian clock and cell cycle at single cell level

• In vitro chronopharmacology of anticancer drug irinotecan
Modified FUCCI reporter system introduced into NIH3T3 cells carrying the Rev-erbα-Venus reporter.

Cell Cycle probes: Crimson, Geminin, mKO2, hCdt1

Clock probes: Rev-erbα, Venus

Feillet et al. PNAS (in press)
1:1 phase-locked Circadian Clock and Cell Cycle
in undisturbed NIH3T3 cells

Circadian Clock period: 18.6 h ± 0.6
Cell Cycle period: 18.2 h ± 0.5

Feillet et al. PNAS (in press)
1:1 phase-locked Circadian Clock and Cell Cycle in undisturbed NIH3T3 cells

Peak transcriptional activity of clock gene Rev-erbα predicts timing of cell division

15% FBS in culture medium

Feillet et al. PNAS (in press)
Cell culture
Circadian Control of Cancerogenesis & Cancer Chronopharmacology

Systems Cancer Chronotherapeutics

Cell culture

2 h

Circadian synchronisation

- 50% FCS or
- Dexamethasone

Samples every 10 min - 4 h for 48-72 h
In vitro-in silico chronopharmacology of anticancer drugs

Cell culture

2 h

Circadian synchronisation

- 50% FCS or
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Samples every 10 min- 4 h for 48-72 h
Genetic clock in human CaCo-2* cell populations

Lévi et al., Annu Rev Pharm Toxicol 2010; 50: 377-421

* Human colorectal cancer cell line: ~ mimicks colon epithelium at confluence
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Ballesta et al., Plos Comput Biol, 2011; e1002143

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A Combined Experimental and Mathematical Approach for Molecular-based Optimization of Irinotecan Circadian Delivery

Variables studied in synchronized and non synchronized Caco-2 cells
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Ballesta et al., Plos Comput Biol, 2011; e1002143
Irinotecan chronotherapeutic model
Molecular PD according to circadian drug timing and drug exposure duration

Ballesta et al., Plos Comput Biol, 2011; e1002143.
Irinotecan chronotherapeutic model
Molecular PD according to circadian drug timing and drug exposure duration

Model validation
• Molecular clock
• Cellular chronoPK
• Cellular chronoPD
• In vitro chronotoxicity

Model adjustment

Ballesta et al., Plos Comput Biol, 2011; e1002143.
Dulong et al., Submitted
Irinotecan chronotherapeutic model
Molecular PD according to circadian drug timing and drug exposure duration

Model validation
- Molecular clock
- Cellular chronopharmacology
- Cellular chronopharmacodynamics
- *In vitro* chronotoxicity

Model adjustment

*Ballesta et al., Plos Comput Biol, 2011; e1002143.*

*Dulong et al., Submitted*
Clock-controlled irinotecan bioactivation and detoxification

Human CaCo-2 cells at confluence

Circadian Time since serum shock onset (h)

Cells exposed to siRNA then synchronized 24 h later with a 2h 50% FCS shock

Dulong et al. Submitted
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Cells exposed to siRNA then synchronized 24 h later with a 2h 50% FCS shock

Dulong et al.
Submitted
Clock-controlled irinotecan bioactivation and cytotoxicity (apoptosis)

Control siRNA

Bmal1 siRNA

Time since irinotecan exposure onset (h)

[SN38]_{in} (nM/10^6 cells)

CT2
CT14
CT20
Clock-controlled irinotecan bioactivation and cytotoxicity (apoptosis)

Control siRNA

Bmal1 siRNA

[SN38]_{in} (nM/10^6 cells)

Time since irinotecan exposure onset (h)

Apoptosis (%)

siRNA control

siRNA Bmal1

CT2

CT14

CT20

Dulong et al.
Submitted
Clock-controlled irinotecan bioactivation and cytotoxicity (apoptosis)

**Model and sensitivity analyses**

- Confirm critical role for clock control of CES2 irinotecan bioactivation
- Add critical role for clock control of UGT1A1 irinotecan detoxification

**Model validation in**

- Other cell lines (ongoing)
- Mice (ongoing)
- Cancer patients (circadian biomarkers)
Clock- Chronopharmacology at cell population level

• Non malignant & cancer cells other than CaCo-2 at confluence?
• Impact of clock-cell cycle coupling?
• Circadian synchronisation?
• Robust chronotherapy model linked to clock markers?
• *In vivo* validation?
Sex and genetic differences in irinotecan chronotolerance in mice: 3 classes

Figure A: Body weight loss for B6D2F1 mice at different Zeitgeber times (h) for females and males.

Figure B: Body weight loss for B6CBAF1 mice at different Zeitgeber times (h) for females and males.

Figure C: Body weight loss for B6D2F1 and C57BL/6 mice at different Zeitgeber times (h) for females.

Figure D: Body weight loss for B6D2F1 and C57BL/6 mice at different Zeitgeber times (h) for males.

Figure E: Body weight loss for B6CBAF1 and B6CBAF1 mice at different Zeitgeber times (h) for females and males.

Li et al. Cancer Res 2013
Sex and genetic differences in irinotecan chronotolerance in mice: 3 classes

Li et al.  
Cancer Res 2013
Sex and genetic differences in irinotecan chronotolerance in mice: 3 classes

![Graphs showing body weight loss over different Zeitgeber times for B6D2F1 and B6CBAF1 strains, with male (♂) and female (♀) mice at different Zeitgeber times (ZT11 and ZT15).](image)
Best for

- C57Bl/6
- B6D2F1
- B6CBAF1

ZT0
- Per2<sup>m/m</sup>

ZT12
- C57Bl/6
- B6D2F1
- B6CBAF1

ZT24/0
- B6CBAF1
Worst for

♂ B6CBAF1
♀ B6CBAF1
♀ Per2<sup>m/m</sup>

Best for

♂ C57Bl/6
♂ B6D2F1
♂ B6CBAF1
♀ Per2<sup>m/m</sup>
♀ C57Bl/6
♀ B6D2F1
♀ B6CBAF1
Worst for

♂ B6CBAF1
♀ B6CBAF1
♀ Per2<sup>m/m</sup>

Best for

♂ C57Bl/6
♂ B6D2F1
♀ Per2<sup>m/m</sup>

♀ C57Bl/6
♀ B6D2F1
♀ B6CBAF1

8 h-range for optimal irinotecan timing (tolerability) according to sex & genotype in mice
8 h-range for optimal irinotecan timing (tolerability) according to sex & genotype in mice

Which of 27 liver or colon circadian gene transcription patterns predict for optimal timing if any?
8 h-range for optimal irinotecan timing (tolerability) according to sex & genotype in mice

Which of 27 liver or colon circadian gene transcription patterns predict for optimal timing if any?
8 h-range for optimal irinotecan timing (tolerability) according to sex & genotype in mice

Which of 27 liver or colon circadian gene transcription patterns predict for optimal timing if any?

Li et al. Cancer Res 2013
A circadian clock transcription model for the personalization of cancer chronotherapy timing

Li et al. Cancer Res 2013
A circadian clock transcription model for the personalization of cancer chronotherapy

Li et al. Cancer Res 2013
Circadian Control of Cancerogenesis & Cancer Chronopharmacology

![Cancer Clock](image_url)
Circadian Control of Cancerogenesis & Cancer Chronopharmacology

- Systematic exploration of role of clock genes in liver and cancers for cell cycle, drug metabolism and chronotherapeutic effects
- Continuous physiology monitoring & dynamic molecular imaging

Per2::luc/Bmal1::luc ± si/shRNA
Rev-Erbα-FUCCI

Per2::luc
Abcb1a::luc
Clock mutant
Cancer prone

Lumicycle
RT-Bio
IVIS Spectrum
Per2 trancription dynamics in liver cancer (HEPA1-6 Per2::luc clone3)

Dex-pulsed cultures (40% confluence)

Counts/Sec

Time (hours)
Per2 transcription dynamics in liver cancer (HEPA1-6 Per2::luc clone3)

Dex-pulsed cultures (40% confluence)

Counts/Sec

Time (hours)

N of photons

Time (days)

10^7 cells sc on day 0 in NMRI mouse

XM Li, V Roche, N Ozturk, M Dumitru, S Dulong
**Per2 transcription dynamics in liver cancer** *(HEPA1-6 Per2::luc clone3)*

**Dex-pulsed cultures (40% confluence)**

- **Counts/Sec**
- **Time (hours)**
- **N of photons**
- **Agent**
- **Luciferin pump**

**Per2 transcription dynamics in mouse liver** *(Per2::luc KI/KI mouse)*

- **Counts/Sec**
- **Time (days)**
- **N of photons**
- **Agent**
- **Luciferin pump**

*XM Li, V Roche, N Ozturk, M Dumitru, S Dulong*
Timing of circadian maximum (acrophase) in rest-activity and skin surface temperature rhythms
10 individual patients before, during and after a 4-day course of chronotherapy
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Roche et al. Chronobiology Intern 2014
Timing of circadian maximum (acrophase) in rest-activity and skin surface temperature rhythms
10 individual patients before, during and after a 4-day course of chronotherapy

Roche et al. Chronobiology Intern 2014
Conclusions

• The Circadian Timing System controls cancer processes and treatment effects through redundant coordinated mechanisms
→ Targeting host clocks can prevent or halt cancer via host mechanisms
→ Targeting tumor clocks could enhance efficacy or cure cancer

• New coordinated \textit{in vitro-in silico-in vivo} cancer chronotherapeutics (Systems Biology - Systems Medicine)

• Mathematical models and dedicated technologies critical for designing & delivering optimal chronotherapy schedule and strategy.