Shift work/LAN, circadian disruption and cancer

- Melatonin
- Clock genes

↓ Oncostatic activity
↑ Tumor promotion

Focus: breast cancer

6.2: Melatonin & Clock genes
Shift work/LAN, circadian disruption and cancer

**Outline: Mechanistic Data**

**6.2: Melatonin & Clock genes**
- Focus: breast cancer

**6.3: LAN-related exposures and key events related to cancer**

LAN = light at night
KC = key characteristics of carcinogens
Outline: Mechanistic Data

Shift work/LAN, circadian disruption and cancer

6.2: Melatonin & Clock genes

6.3: LAN-related exposures and key events related to cancer

6.4: Other mechanisms (vitamin D, sleep, meal timing)
Outline: Mechanistic Data

Shift work/LAN, circadian disruption and cancer

Circadian disruption
- Melatonin
- Clock genes

Biological effects
- ↓ Oncostatic activity
- ↑ Tumor promotion

Cancer
- Focus: breast cancer

6.2: Melatonin & Clock genes
Breast Cancer and Melatonin

Is LAN a possible risk factor for breast cancer?

- Breast cancer risk has ↑ as societies industrialize
- LAN has also ↑ as societies industrialize
- Known risk factors account for <50% of cases
- LAN ↓ nocturnal melatonin production
- Melatonin inhibits breast tumor growth
- Proposed mechanism (melatonin hypothesis)

LAN → ↓ nocturnal melatonin production → ↑ estrogen → ↑ turnover epithelial stem cells → ↑ breast cancer risk
Types of evidence to evaluate the melatonin hypothesis

- Human cancer studies of night shift work (Section 3)
  - Originally thought to be a surrogate for extreme LAN
- Human cancer studies of LAN exposures (Section 3)
- Human studies of melatonin (or proxies) and cancer risk
  - Cohort studies of shift workers
  - Visually impaired/blind populations
- Experimental studies of melatonin and cancer growth
- Mechanistic studies of melatonin
Human studies: melatonin and cancer risk

- Shift workers
  - Some evidence of inverse association with breast cancer
  - Stronger evidence in post-menopausal women (2 independent cohorts)
  - Limited number of studies, inconsistencies
Human studies: melatonin and cancer risk

• Shift workers
  – Some evidence of inverse association with breast cancer
  – Stronger evidence in post-menopausal women (2 independent cohorts)
  – Limited number of studies, inconsistencies

• Totally blind/visually impaired
  – Melatonin is not suppressed by LAN in totally blind people
  – Melatonin rhythms: free running/abnormally entrained
  – Breast cancer: Inverse association with blindness and degree of visual impairment (6 studies)
  – Prostate cancer: lower risk (non-significant) (2 studies)
LAN, Melatonin and Cancer in Rodents

LAN suppresses melatonin and promotes tumor growth

MLT = melatonin
LAN, Melatonin and Cancer in Rodents

Exogenous melatonin suppresses tumor growth

MLT = melatonin
a = Blood collected from humans at night (no LAN) or synthetic MLT added to rat blood
LAN, Melatonin and Cancer in Rodents

Exogenous melatonin suppresses tumor growth

-↓ cAMP
-↓ Linoleic acid uptake
-↓ 13-HODE
-↓ AKT signaling
-↓ Warburg effect
-↓ Tumor DNA synthesis

LAN

Exogenous MLT
- Oral
- Injected
- Blood a

↓ Chemically induced tumors
↓ Tumor implants

cAMP = cyclic adenosine monophosphate
13-HODE = 13-hydroxyoctadecadienoic acid
MLT = melatonin

a = Blood collected from humans at night (no LAN) or synthetic MLT added to rat blood
Low MLT blood or high MLT blood + MLT receptor antagonist do not suppress tumor growth

LAN, Melatonin and Cancer in Rodents

MLT = melatonin
a = Blood collected from humans at night (no LAN) or synthetic MLT added to rat blood
## Melatonin and Cancer

### Melatonin is oncostatic via multiple pathways

<table>
<thead>
<tr>
<th>Mechanism/Pathway</th>
<th>Key events and effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen receptor &amp; enzyme modulator</td>
<td>↓ Estrogen receptor (ERα) activation &amp; expression</td>
</tr>
<tr>
<td></td>
<td>↓ Estradiol</td>
</tr>
<tr>
<td>Antioxidant, oxidative stress response</td>
<td>↓ ROS, NOS</td>
</tr>
<tr>
<td></td>
<td>↑ GSH, SOD, catalase</td>
</tr>
<tr>
<td>Immune activation</td>
<td>↑ NK cells, leukocytes, monocytes, cytokines, IFN-γ, TNFα</td>
</tr>
<tr>
<td></td>
<td>↑ Immunosurveillance</td>
</tr>
<tr>
<td>Cell cycle, differentiation &amp; apoptosis</td>
<td>↑ G1, cell cycle length, p53, p21, caspases, differentiation, apoptosis</td>
</tr>
<tr>
<td></td>
<td>↓ Cyclin D1, cell proliferation</td>
</tr>
<tr>
<td>Telomerase inhibition</td>
<td>↓ hTERT, estradiol-induced telomerase activity</td>
</tr>
<tr>
<td></td>
<td>↓ Number of neoplastic cell replication cycles</td>
</tr>
<tr>
<td>Angiogenesis inhibition</td>
<td>↓ VEGF, HIF-1α, ROS, neovascularization</td>
</tr>
<tr>
<td>Metastasis inhibition</td>
<td>↓ response to estradiol, cell invasiveness/metastasis</td>
</tr>
<tr>
<td></td>
<td>↑ E-cadherin, β₁-integrin, MT1 receptor</td>
</tr>
<tr>
<td>Fatty acid uptake and metabolism</td>
<td>↓ Linoleic acid uptake, 13-HODE</td>
</tr>
<tr>
<td></td>
<td>↓ EGFR/MAPK activity</td>
</tr>
</tbody>
</table>

Source: Mediavilla et al. 2010
Circadian Disruption Theory

LAN/Shift work effects > melatonin suppression

• Core clock genes
  – Control expression of 2% -10% of the genome
  – Mutations/deregulated expression common in cancers
  – SNPs: increased risk of breast and other cancers

SNPs = single nucleotide polymorphisms
Circadian Disruption Theory

LAN/Shift work effects > melatonin suppression

• Core clock genes
  – Control expression of 2% -10% of the genome
  – Mutations/deregulated expression common in cancers
  – SNPs: increased risk of breast and other cancers

• Desynchronizes central clock/SNS/peripheral clock
  – Disrupted cell signaling pathways and regulatory circuits
  – Loss of cell cycle control and altered metabolism
  – ↑ Cell proliferation and ↓ apoptosis
  – ↓ Tumor suppression and DNA repair

SNS = sympathetic nervous system
## Core Clock Genes: Genetic Models

### Mutant mice exhibit a cancer-prone phenotype and accelerated tumor growth

<table>
<thead>
<tr>
<th>Mutant gene</th>
<th>Tumors</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bmal1</em> or <em>Per2</em></td>
<td>Lung</td>
<td>Accelerated tumor growth/progression, ↑ c-Myc, metabolic dysregulation</td>
</tr>
<tr>
<td><em>Bmal1</em> or <em>Per2</em></td>
<td>Colon</td>
<td>Accelerated tumor growth in vivo/in vitro</td>
</tr>
<tr>
<td><em>Bmal1, Per1, Per2, Cry1, and Cry2</em></td>
<td>Liver, Ovarian Lymphoma</td>
<td>Increased incidence of spontaneous and radiation-induced tumors</td>
</tr>
<tr>
<td><em>Per2, Cry1, and Cry2</em></td>
<td>Liver Bile duct</td>
<td>Developed 4-8X more tumors than WT mice</td>
</tr>
</tbody>
</table>
Outline: Mechanistic Data

Shift work/LAN, circadian disruption and cancer

6.2: Melatonin & Clock genes
- Melatonin
- Clock genes
- Oncostatic activity
- Tumor promotion
Focus: breast cancer

6.3: LAN-related exposures and key events related to cancer

LAN = light at night
KC = key characteristics of carcinogens
LAN/Shift Work: Biological Effects

LAN/Shift work are associated with KC/other effects

Human studies: Shift work

- ↓ DNA repair
- ↑ Genomic instability
- ↑ Oxidative damage
- ↑ Chronic inflammation & immune suppression
- Epigenetic alterations
- Altered cellular metabolism
- Altered hormone rhythms & signaling

Rodent studies:
- Jet lag/Shift work
- LAN

KC = Key characteristics of carcinogens
The circadian clock is regulated at the epigenetic level.

Human studies: Shift work
- ↓ DNA repair
- ↑ Genomic instability
- ↑ Oxidative damage
- ↑ Chronic inflammation & immune suppression
- Epigenetic alterations
- Altered cellular metabolism
- Altered hormone rhythms & signaling

Rodent studies: Jet lag/Shift work

Rodent studies: LAN
- Tumors have aberrant clock gene methylation patterns
- Altered methylation patterns in shift workers (clock and genome wide)
- LAN inhibits DNA methyltransferase
- CLOCK: intrinsic histone acetylase (HAT) activity
- May account for MLT oncostatic properties
LAN/Shift Work: Biological Effects

Melatonin regulates sex hormone rhythms

Human studies: Shift work
- ↓ DNA repair
- ↑ Genomic instability
- ↑ Oxidative damage
- ↑ Chronic inflammation & immune suppression
- Epigenetic alterations
- Altered cellular metabolism
- Altered hormone rhythms & signaling

Rodent studies:
- Jet lag/Shift work
- LAN

Human studies:
- ↑ Gonadotropins
  - Testosterone
  - Progesterone
  - Estrogens
- Altered estrous function & rhythm (rodents)
- ↑ Estrogen & metabolites (shift workers)
- Relationship to melatonin uncertain
Outline: Mechanistic Data

Shift work/LAN, circadian disruption and cancer

6.2: Melatonin & Clock genes

6.3: LAN-related exposures and key events related to cancer

6.4: Other mechanisms (vitamin D, sleep, meal timing)

LAN = light at night
KC = key characteristics of carcinogens

Circadian disruption ➔ Biological effects ➔ Cancer

- Melatonin
- Clock genes

↓ Oncostatic activity
↑ Tumor promotion

Focus: breast cancer

↑ Biological effects (CD, KC)

Other LAN/shift work exposures
Night shift work is a complex exposure scenario
Vitamin D regulates many of the same biological processes as melatonin

- 90% from sunlight exposure
- Regulates > 2000 genes
  - Metabolism
  - DNA repair
  - Antioxidant activity
  - Immune function/inflammation
  - Cell proliferation/differentiation
- Deficiency and cancer
  - Risk factor in human cancers
  - Role in breast cancer uncertain
    - VDR knockouts: ↑ preneoplasia
    - VDR SNPs: ↑ breast cancer risk

VDR = vitamin D receptor
SNPs = single nucleotide polymorphisms
The sleep/wake cycle is bidirectionally associated with the circadian system

- Misalignment with LD cycle
- Disruption affects function of multiple systems:
  - Inflammation and immune response
  - Metabolic (insulin, glucose, leptin, ghrelin)
  - Cellular (DNA damage/oxidative stress, epigenetic)
  - Neuroendocrine
- Role in breast cancer uncertain
  - Mixed results from human studies
  - Plausible mechanisms
  - More studies needed

LD = daily and seasonal light:dark cycle
Meal timing is an important non-photic zeitgeber

- Peripheral clock entrainment
- Gene expression/biomarkers
  - Glucose homeostasis & energy metabolism
  - Inflammation & immune function
  - Tyrosine kinase signaling
  - DNA damage checkpoints
  - C-reactive protein
  - Oxidative stress
- Role in cancer
  - Restricted feeding ↓ tumor growth
  - After 10:00 PM ↑ breast cancer
Summary

- **Melatonin and clock genes**
  - Maintain tissue and cellular homeostasis
  - Multiple oncostatic pathways

- **LAN, shift work, jet lag induce circadian disruption**
  - Melatonin suppression
  - Altered clock gene and clock controlled gene expression
  - Associated with multiple key characteristics of carcinogens

- **Complex exposures/interactions**
  - Sunlight and vitamin D
  - Sleep disruption
  - Meal timing
Clarification questions?
Reviewer Comments

– Comment on whether the information provided in the Mechanistic and Other Relevant Data section is clear, technically correct, and objectively presented.

– Identify any information that should be added or deleted.

– Provide any scientific criticisms of NTP’s synthesis of the mechanistic data for assessing effects of night shift work and light at night.

– Comment on whether the summary captures the key information for each topic.