Mechanistic and Other Relevant Data

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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
Outline: Mechanistic Data

Shift work/LAN, circadian disruption and cancer

Circadian disruption
- Melatonin
- Clock genes

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Biological effects

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6.2: Melatonin & Clock genes

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
- Melatonin
- Clock genes
- ↓ Oncostatic activity
- ↑ Tumor promotion
- Focus: breast cancer

6.3: LAN-related exposures and key events related to cancer
- ↑ Biological effects (KC)

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

- Other LAN/shift work exposures
Shift work/LAN, circadian disruption and cancer

Outline: Mechanistic Data

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
Melatonin Hypothesis

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
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• 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

LAN

↑ Spontaneous tumors
↑ Chemically induced tumors
↑ Tumor implants
↓ Endogenous MLT

MLT = melatonin
LAN, Melatonin and Cancer in Rodents

Exogenous melatonin suppresses tumor growth

LAN

↓ Chemically induced tumors

↓ Tumor implants

Exogenous MLT
- Oral
- Injected
- Blood a

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

\(cAMP = \text{cyclic adenosine monophosphate}\)
\(13\text{-HODE} = 13\text{-hydroxyoctadecadienoic acid}\)
\(MLT = \text{melatonin}\)
\(^a = \text{Blood collected from humans at night (no LAN) or synthetic MLT added to rat blood}\)
LAN, Melatonin and Cancer in Rodents

Low MLT blood or high MLT blood + MLT receptor antagonist do not suppress tumor growth

MLT= melatonin
a = Blood collected from humans at night (no LAN) or synthetic MLT added to rat blood
## 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 ▼ Estradiol</td>
</tr>
<tr>
<td>Antioxidant, oxidative stress response</td>
<td>▼ ROS, NOS ▲ GSH, SOD, catalase</td>
</tr>
<tr>
<td>Immune activation</td>
<td>▲ NK cells, leukocytes, monocytes, cytokines, IFN-γ, TNFα ▲ Immunosurveillance</td>
</tr>
<tr>
<td>Cell cycle, differentiation &amp; apoptosis</td>
<td>▲ G1, cell cycle length, p53, p21, caspases, differentiation, apoptosis ▼ Cyclin D1, cell proliferation</td>
</tr>
<tr>
<td>Telomerase inhibition</td>
<td>▼ hTERT, estradiol-induced telomerase activity ▼ 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 ▲ E-cadherin, β1-integrin, MT1 receptor</td>
</tr>
<tr>
<td>Fatty acid uptake and metabolism</td>
<td>▼ Linoleic acid uptake, 13-HODE ▼ EGFR/MAPK activity</td>
</tr>
</tbody>
</table>

Source: Mediavilla et al. 2010
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
• 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
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</em>, <em>Per1</em>, <em>Per2</em>, <em>Cry1</em>, and <em>Cry2</em></td>
<td>Liver, Ovarian Lymphoma</td>
<td>Increased incidence of spontaneous and radiation-induced tumors</td>
</tr>
<tr>
<td><em>Per2</em>, <em>Cry1</em>, and <em>Cry2</em></td>
<td>Liver, Bile duct</td>
<td>Developed 4-8X more tumors than WT mice</td>
</tr>
</tbody>
</table>
Shift work/LAN, circadian disruption and cancer

Outline: Mechanistic Data

6.2: Melatonin & Clock genes

Focus: breast cancer

- Melatonin
- Clock genes

↓ Oncostatic activity
↑ Tumor promotion

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

KC = Key characteristics of carcinogens

Rodent studies: LAN

Rodent studies: Jet lag/Shift work
The circadian clock is regulated at the epigenetic level

LAN/Shift Work: Biological Effects

Human studies: Shift work

- ↓ DNA repair
- ↑ Genomic instability
- ↑ Oxidative damage
- ↑ Chronic inflammation & immune suppression
- Epigenetic alterations
- Altered cellular metabolism
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Rodent studies: Jet lag/Shift work

- 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

Rodent studies: LAN
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

Rodent studies:
- LAN
Shift work/LAN, circadian disruption and cancer

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

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Night Shift Work: Co-exposures

Night shift work is a complex exposure scenario

- Light at Night
- Sleep Disruption
- Altered Meal Timing
- Vitamin D
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
Night Shift Work and LAN: Mechanistic Data

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.