MECHANISMS OF HORMONAL CARCINOGENESIS

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WHAT CAUSES CANCER?

- Understanding what causes cancer is essential to prevent, detect and successfully treat this disease.

**Genes** a person inherits and **exposures** to factors that can cause mutations and/or epigenetic changes

- Increased cell proliferation and survival; reduced cell differentiation and DNA damage repair

- Development of cancer

Normal cell → Cancer cell
Carcinogenesis

- Causes of most cancers are not known
- Most cancers probably are multifactorial in origin
- Several carcinogenic agents identified, but many probably remain unidentified.
  - Exposure levels to most industrial, agricultural, and household chemicals low, but long-lasting
  - Long lag phase between exposure and detection of cancer
EXPOSURES LINKED TO INITIATION OF CANCER

- Radiation
- **Tobacco smoke**
- Carcinogens
- **Hormones/ hormone mimicking compounds**
- Viruses
- Heavy metals (cadmium, arsenic, nickel, etc.)
- Diet
- Etc.

Changes in **oncogene** (induce cell proliferation, inhibit differentiation) and **tumor suppressor gene functions** (control cell cycle progression, induce apoptosis, repair DNA damage):

- These changes caused by mutations and/or epigenetic modifications
Mechanisms that initiate cancer

- **MUTATIONS:**
  - DNA is damaged, and it produces a “sick” protein

- **EPIGENETIC CHANGES:**
  - DNA is intact, but it either produces too little or too much protein.
Endocrine system

1. Pineal gland (melatonin)
2. Hypothalamus (Releasing hormones for: Thyrotropin, Gonadotrophin, Growth hormone and Corticotrophin, Somatostatin) and Pituitary gland (e.g., Prolactin, FSH, LH)
3. Thyroid gland (T3, T4, calcitonin)
4. Thymus
5. Adrenal gland (Cortisol, aldosterone, androgens)
6. Pancreas (Insulin, Glucagon) and Liver (IGF)
7. Ovary (estrogens)
8. Testis (androgens)

They secrete steroidal or protein-based hormones

Other tissues:
- Skin (vitamin D)
- Adipose tissue (leptin, estrogens)
Two types of hormones

- **Steroid hormones** bind to nuclear receptors, which serve as on-off switches → development and differentiation of reproductive tissues, brain, bone and skin.

- **Protein/amino acid hormones** bind to receptor sites on cell membrane and use second messengers that activate or deactivate enzymes which modify protein pathways within the cell.
Estrogen Signaling

ER effects on different cell types


Plasma membrane

Endothelial cell
Vasorelaxation

Neuron
Neuroprotection

Breast cancer cell
Cell cycle stimulation

Osteoblast
Cell proliferation/differentiation
Bone conservation
Estrogen Receptors

- \( \text{ER}\alpha \): induces proliferation
- \( \text{ER}\beta \): inhibits actions of \( \text{ER}\alpha \)
  - Other functions?

http://www.bio.cmu.edu/Courses/BiochemMols/ER/#ERchime
Estrogens increase breast, uterine/endometrial cancer risk

- In the Western world, 1 of every 8 women will develop breast cancer during their life-time.
- Several risk factors for breast cancer have been identified.
- Many of them relate to sex steroids:
  - sex of the affected individuals (women)
  - parity
  - late age at first pregnancy
  - early age of menarche and late age of menopause
- However, the known risk factors account for only 20-25% of disease occurrence!
Hormonal carcinogenesis

- Hormones or hormone-like substances can either
  - Initiate carcinogenesis
  - Promote tumor growth

- High hormone levels
- High rate of cell proliferation
- Increased risk of mutations?
- Increased cancer risk
- Reduced cancer risk
Evidence that hormones can initiate cancer

Potent estrogens = Induce mutations?

- Supportive data obtained in in vitro systems (Russo et al.)
- Estrogens induce tumors in some animal models (pituitary, mammary gland, ovaries)

Weak estrogens = no harm?

Is DNA repair system intact?
TIMING OF EXPOSURE

• The breast undergoes extensive growth during:
  
  – **Fetal life**: The mammary fat pad is being formed and the primary epithelial tree begins to grow.
  
  – **Puberty**: Rapid growth of the mammary gland, both epithelium and stroma.
  
  – **Pregnancy**: Mammary gland proliferates extensively, followed by alveolar differentiation.

• High levels of hormones and growth factors program/re-programming the mammary gland at fetal period, puberty and pregnancy.
Maternal dietary exposures during pregnancy affect female offspring’s breast cancer risk.

**DMBA-induced mammary tumor incidence:**
- *in utero* n-6 PUFA corn oil exposure
- High n-6 PUFA
- Low n-6 PUFA

**Total number of tumors/group**

- Control
- Oat Flour
- Whole Wheat
- Defatted Flax

**Number of tumors/rat**

- CONTROL
- MODERATE ALCOHOL
- HIGH ALCOHOL

**Maternal diet**
- High fat (39%) PUFA diet
- High estrogenic environment

**Fiber**

**Alcohol**
High birth weight is associated with increased premenopausal breast cancer risk in numerous studies.

- May reflect high in utero estrogen, leptin, insulin, IGF-1, etc. environment.


*Hilakivi-Clarke & de Assis, TEM 2006*
High birth weight: Experimental Design

- **Control diet** (16% fat)
  - Extent of pregnancy

- **Obesity-inducing diet** (45% fat)
  - Extent of pregnancy

**Pregnant Sprague-Dawley Rats**

**DMBA exposure**

**Measure:**
- Pups birth weight

**Collect:**
- Serum
- Mammary glands (3w and 8w)

**Measure:**
- Mammary tumorigenesis

**50-day-old female offspring**

*International J Cancer, 2006*
Birth weight is significantly elevated in the offspring of dams fed an obesity-inducing diet (OID, 45% fat) during pregnancy.
Exposure to obesity-inducing diet during pregnancy increases circulating leptin levels in mothers.

Leptin

Estradiol

IGF-1

Leptin potentiates ER-α activation and increases aromatization.
DMBA: a model to study estrogen-dependent tumorigenesis

- 7,12-dimethylbenz[a]anthracene (DMBA) is a polycyclic aromatic hydrocarbon (PAH).
- PAHs may play an etiological role in some human breast cancers.

DMBA-induced mammary tumors develop by a multi-step process:

- **STEP 1:** DMBA binds to DNA causing a biochemical lesion
- **STEP 2:** Initiating mutations occur (Ras mutations in 20-30% of tumors)
- **STEP 3:** Cell proliferation/growth → tumor

From Russo and Russo

- Tamoxifen-responsive
- Histopathology of mammary tumors resemble those of human breast cancers.
High birth weight increases mammary gland tumorigenesis

**tumor incidence**

Log rank test: p=0.38

**tumor latency**

t-test: p=0.026

**tumor volume**

Two-way RM ANOVA: p<0.001

**tumor multiplicity**

t-test: p=0.17
High birth weight alters mammary gland protein expression

High birth weight is associated with reduced ER-α expression in the developing mammary gland
High birth weight alters mammary gland protein expression

High birth weight is associated with increased levels of activated MAPK in the adult mammary gland.
Low birth weight and mammary cancer
(Ozanne et al., Carcinogenesis 2006)

- Low birth weight was induced by feeding pregnant dams a low protein diet (8% energy from protein versus 20% in the control diet).
- Low birth weight increased incidence of early onset mammary cancers.

<table>
<thead>
<tr>
<th>Weeks after treatment</th>
<th>Control (tumor/non-tumor)</th>
<th>LP (tumor/non-tumor)</th>
<th>Relative Risk of Incidence (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-16</td>
<td>8/40</td>
<td>17/31</td>
<td>2.13 (1.015, 4.450)</td>
<td>0.046</td>
</tr>
<tr>
<td>17-32</td>
<td>17/23</td>
<td>15/16</td>
<td>1.14 (0.6, 1.90)</td>
<td>0.642</td>
</tr>
</tbody>
</table>
Low birth weight and mammary cancer: mechanisms

- Low birth weight is associated with increased expression of insulin receptor (IR) and IGF-1R.
  - The increase is seen only after puberty onset
Inherited genetic material is re-programmed by epigenetic means during fetal period.

Epigenetic modifications include (1) methylation of CpG sites within genomic DNA, (2) modifications to histone by acetylation, mono-, di-, tri-methylation, phosphorylation, etc, (3) imprinting, and (4) miRNAs: associate with histone modifiers to form silent heterochromatic regions.

The plasticity of the epigenome during fetal period makes it susceptible to even transient exposures (for example, to hormones or dietary factors that modify hormone levels).

ALTERED SUSCEPTIBILITY TO BREAST CANCER
Altered *in utero* environment induces epigenetic changes?

- **Examples**
  - Expression of $A^{vy}$ allele in Agouti mice causes **yellow coat color**.
  - Maternal exposure to **methyl-supplemented diet** silences the expression of $A^{vy}$ allele and reduces the number of offspring which are yellow.
Maternal Supplements with Folic acid Genistein

LTR Hypomethylated

• High risk cancer, diabetes, obesity
• Reduced lifespan


LTR Hypermethylated

• Lower risk of cancer, diabetes, obesity
• Prolonged life

Yellow Mouse

Agouti Mouse
In utero exposures, alterations in DNA methylation and cancer risk

- Maternal exposure to Bisphenol A induces hypermethylation of PDE4D4 (enzyme responsible for cyclic AMP breakdown) and increases prostate cancer risk (Ho et al., Cancer Research 2006).

- Maternal exposure to synthetic estrogen diethylstilbestrol (DES) induces persistent expression of c-fos and lactoferrin (hypomethylation), and repression of Hoxa-10 and Hoxa-11 (by histone modifications) in female uterine track in mice, and increases uterine cancer risk (Newbold et al.).
**Early life** exposure to DES and altered uterine/urogenital cancer risk

- Prenatal exposure to DES increases the risk of developing tumors in uterine myometrium in Tsc-2 mice (Cook et al., PNAS 2005).
  - Changes in ER expression

- Postnatal DES treatment exacerbates urogenital carcinogenesis in female mice (Waalkes et al., Cancer Res. 2006).
  - Changes in ER expression
High birth weight alters mammary gland cell proliferation (PCNA)

21 days

Control                        High Birth Weight

Proliferation Index

0 20 40 60 80 100

All epithelial structures

t-test: p<0.05

Terminal End Buds

Control                        High Birth Weight

Proliferation Index

0 20 40 60 80

All epithelial structures

t-test: p<0.05

50 days

Control                        High Birth Weight

Proliferation Index

0 20 40 60 80 100

All epithelial structures

t-test: p>0.05

Terminal End Buds

Control                        High Birth Weight

Proliferation Index

0 20 40 60

All epithelial structures

T-test: p<0.05

All epithelial structures

T-test: p>0.05

High birth weight alters mammary gland cell proliferation (PCNA)
High birth weight alters mammary gland morphology and number of undifferentiated structures

21 days

Control

High Birth Weight

50 days

Control

High Birth Weight

Number of TEBs

Epithelial Density

t-test: p=0.15

t-test: p=0.12

t-test: p<0.05

Epithelial density (score 0-5)

0 1 2 3 4 5 6

*
"Tumor suppressor" cells

In utero exposures induce epigenetic changes that promote cell proliferation (more targets for cancer initiation) and inhibit differentiation (less tumor suppressor function)
EPIGENETIC CHANGES*: Affect mammary stem cell self-renewal and fate

- Increased
- Reduced

INCREASED BREAST CANCER RISK
- Carcinogens, radiation, diet, hormones, etc.

REDUCED BREAST CANCER RISK
- Pregnant
- Mother and daughter
- Prepubertal

EXPOSURE:
- Estradiol
- Genistein
- High fat diet
- Obesity, etc.

Number of TEBs ↑

Susceptibility to breast cancer:
- Increased
- Reduced

EXPOSURE:
- Estradiol
- Genistein
- High fat diet
- Obesity, etc.

Number of TEBs ↓

*Genes affected:
- Regulate cell proliferation
- Inhibit differentiation
- Promote survival
- Repair DNA damage

* Direction of changes depends on timing of exposure
SMOKING CAUSES CANCER

- 85% of lung cancer patients are smokers (past or current)
- 10-15% of smokers develop lung cancer during their life time

- Why some people develop cancer, and some do not?
Hormonal carcinogenesis: timing

- Exposure to hormones or hormone mimicking compounds during tissue specific critical development periods alter later susceptibility to develop cancer.
  - by causing epigenetic modifications in gene expression that regulate stem cell self-renewal and fate.
Dietary factors that modify the epigenome: reverse the increase in cancer risk?

• Dietary factors which induce epigenetic changes, may reverse the increase in cancer risk induced by early life hormonal exposures
  – By inducing methylation (silencing gene expression)
    • folic acid
    • soy isoflavones
  – By reversing methylation (activating genes)
    • epigallocatechin gallate in green tea
  – By modifying chromatin structure
    • compounds in cruciferous vegetables

Sources of Folic Acid
- Liver
- Yeast
- Nuts
- Dried beans
- Whole grains
- Spinach and other leafy greens
- Oranges
- Avacados

Source: The Nutrition Bible
THANK YOU!