The Developmental Basis of Disease and Dysfunction: Environmental Exposures and Animal Models

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Objectives

- Understand the new paradigm of Developmental Basis of Disease and the role of environmental exposures.
- Understand the status of this paradigm in animal models.
All complex diseases are the result of:

- Gene-Environment Interactions over Time!

- Recent “epidemics” of chronic diseases like diabetes, childhood asthma, ADHD, obesity… must be due to environmental, dietary and behavioral changes.
Fetal Origin of Adult Disease: The Barker Hypothesis

- 1989 David Barker found an inverse relationship between birthweight and death from heart disease in England and Wales.
- Studies confirmed by “Dutch Hunger Winter” when food supplies to occupied Netherlands were cut off by Nazis. Individuals born during this time had high incidence as adults of insulin-resistance.

Fetal Origin of Adult Disease (FEBAD) confirmed for
- Coronary heart disease
- Hypertension
- Type II diabetes

Cheryl Walker
Question…. 

- If over or under nutrition can alter developmental programming resulting in increased susceptibility to disease…. 

Can developmental exposure to environmental chemicals do the same thing…. without altering body weight?
The in utero/developmental period is a sensitive window for environmental exposures...death, malformations, low birth weight, functional changes.

In utero/developmental exposure to environmental agents at low environmentally relevant exposures causes a functional change due to altered gene expression altering signal transduction pathways.

The functional change is due to altered programming—a lifelong change resulting from a alteration in gene expression, due to altered imprinting or chromatin structure during development (Epigenetics).

This functional change can result in increased susceptibility to disease later in life.
Why is the developmental period sensitive to environmental chemicals? “The Fragile Fetus”

- The developing organism (fetus and neonate) is extremely sensitive to perturbation by chemicals because:
  - Tissues/organs forming
  - DNA repair
  - Liver metabolism
  - Immune system
  - Blood/brain barrier
  - Detox enzymes
  - ↑ metabolic rate
  - Epigenetic marks set

Organ development proceeds via an intricately orchestrated, temporal pattern of gene expression that is specific to the developing tissue. As a result, toxic exposures that perturb gene expression may have unique effects in the developing tissue or organ.

- Epigenetic marks set

(Prof. Howard Bern, 1992)
Issues in Developmental Basis of Disease Studies

- Type, timing, dose and duration of exposure
- Identification of critical developmental windows (pre- and post-natal)
- Interaction with other, preexisting genetic factors
- Latency between exposure and adverse health outcome
- Transgenerational effects
- Molecular mechanism
- Potential impact of interventions (prevention, reversal)
Environmental Agents Alter Signaling

Nonmonotonic curves are common

adapted from Welshons et al. 2003

Pete Myers
Initial Strategy/Approach

- Animal models to enable direct experimental approaches

- *In utero/developmental exposure* to environmental agent at environmentally relevant concentrations

- Measure onset of disease/dysfunction or exacerbation disease (puberty to adult)

- Measure and correlate gene expression or other cellular changes during development to those in adult diseased tissues
Approach II (Animal Model)

- Show *cause and effect* relationship between *in utero* exposure, altered gene expression and adult disease.

- Show *mechanism* of altered gene expression---pathway to altered gene expression including methylation and other epigenetic changes.

- Develop biomarkers of exposure and susceptibility...Use them in human studies.

- Develop intervention and prevention strategies.
Developmental Basis of Disease: Disease Focus

- **Reproductive/Endocrine**
  - Breast/prostate cancer
  - Endometriosis
  - Polycystic ovary syndrome
  - Fertility
  - Diabetes/metabolic syndrome
  - Puberty
  - Obesity

- **Brain/Nervous System**
  - Alzheimer’s disease
  - Parkinson’s disease
  - ADHD

- **Pulmonocardiovascular**
  - Atherosclerosis
  - Asthma
  - Chronic obstructive pulmonary disease
  - Heart disease/hypertension

- **Immune/Autoimmune**
  - Systemic/tissue specific autoimmune disease
  - Immunosuppression
Developmental Basis of Disease: Environmental Stressor Focus

- Bisphenol A/Environmental Estrogens
- Tributyl Tin
- Phthalates
- DES
- Genistein
- Dioxin/PCBs
- Atrazine
- ETS/ Air Pollution
- Organochlorines/Organophosphates
- Methylmercury/Lead/arsenic
- LPS
- Vinclozolin
- PBDEs
Uterine Leiomyoma

Walker and Stewart
Science 2005

- Most common tumor of women
- Number 1 indication for hysterectomy in the US, accounting for >2000,000 of these surgeries annually
- Hormone dependent requiring estrogen for growth (Cheryl Walker)
The Developmental Basis of Uterine Leiomyoma: Role of Tumor Suppressor Gene Penetrance

- **Tumor:** Uterine Leiomyoma
- **Tumor Suppressor Gene:** TSC2
- **Model:** Eker rat
- **Environmental Agent:** Exposure to the xenoestrogen DES

**Experimental Design:**

- **Inject with 10µg DES or vehicle**
- **Postnatal days 3, 4, 5**
- **Sacrifice 5 mos, 16 mos.**

**Groups:**

- Carrier (Tsc-2^{EK/+}) + DES
- Carrier (Tsc-2^{EK/+}) + Vehicle
- Wildtype (Tsc-2^{+/+}) + DES
- Wildtype (Tsc-2^{+/+}) + Vehicle
### Developmental DES Exposure Increases Tumor Incidence, Multiplicity and Size in Genetically Susceptible Animals.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>N of rats</th>
<th>% Tumor Incidence</th>
<th>Multiplicity (mean no. of tumors/rat)</th>
<th>Size (cm³) Mean ± S.E.M.</th>
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<td>64</td>
<td>0.82</td>
<td>2.3 ± 1.1</td>
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<tr>
<td></td>
<td>DES</td>
<td>24</td>
<td>92*</td>
<td>1.33*</td>
<td>10.5 ± 2.7*</td>
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<tr>
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<td>DES</td>
<td>34</td>
<td>0</td>
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</tr>
</tbody>
</table>

**Developmental reprogramming of estrogen responsiveness**

Cook et al. PNAS 2005
Developmental Re-programming of Estrogen Responsiveness in DES Females

- Target myometrial cells in DES animals hyper-responsive to (low) estrogen levels
- Not observed in liver, which is fully developed in neonates
- Estrogen receptor levels unchanged
- Developmental exposure had reprogrammed estrogen responsiveness
Window of Susceptibility to Developmental Programming: When does it Close?

Uterine mesenchyme segregates into 3 layers: inner, middle, outer

Uterine glands present in stroma

- DES d3-5
- DES d10-12
- DES d17-19
What Defines the Window of Susceptibility in the Uterus?

Uterus is susceptible to developmental programming during time it is normally maintained in an “estrogen naïve” state.
Developmental Exposures and Disease: What’s the Mechanism?

We Know That…

- Many environmental chemicals act via altering gene expression. (Some are hormone mimics)
- Tissue specific alteration of gene expression alters the function of the tissue resulting in increased susceptibility to disease.
- The changes in gene expression are long lasting (irreversible) and may even be transmitted to the next generation.

These data suggest the mechanism responsible for the environmental component of disease is EPIGENETICS.
Same Genotype, Different Epigenotype
Summary

- All complex diseases are due to gene-environment interactions over time.
- Environmental agents act via altering gene expression via epigenetics.
- The most sensitive period for environmental exposures is development.
- Developmental exposures, in animals, results in decreased sperm counts, infertility, prostate cancer, “testicular dysgenesis syndrome”, mammary cancer, obesity, neurodegenerative disease and immune diseases……
- Significance to human health and disease still undetermined.
- Biomarkers of exposure and susceptibility needed.
- Human studies needed.
Take Home Message

- Physicians and epidemiologists need to consider not only that environmental chemicals can cause diseases but that the exposures may have occurred early during development.

- There are animal data...supporting examination of this paradigm in humans.

- To be successful will require interactions and collaborations between scientists doing animal and those doing human studies.
  - Development of markers of developmental exposures
  - Development of markers of functional changes