Transplacental and developmental genotoxicology: complex dynamic of carcinogenesis

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Carcinogenesis research involves multidisciplinary and international studies using large datasets and fast data exchange.
The result is a "noosphere" with a unique potency to integrate and interpret collected knowledge.
Despite invested efforts, cancer incidence is on the rise.

It is particularly alarming in children and adolescents !!!
• Mutation
• Structural rearrangement of chromosomes
• Aneuploidy
• Methylation; reversible
• Mitogen
Intrauterine development and early childhood may be critical for elucidating the cancer aetiology.
It seems that all cancers are oestrogen-positive.

Traditional concept of sex hormones has to be changed.
Xenoestrogens do not follow known genotoxic pathways due to their specific molecular characteristics.
Estrogen

• Evolutionary old molecule present in animals and plants
• endocrine, paracrine agent and neurotransmitter
• modulates development of organs during intrauterine life via receptors and postnatal maturation (bones)
• In non-physiological levels it is mutagen, aneugen, demethylating agent
• it is involved in number of biological pathways - from GABA synthesis in brain to synthesis of membranes in fungi
ESTROGEN RECEPTORS

Alpha

Beta

GPR 30
• Number of chemical agents synthesized by our civilization are estrogen like or have impact on aromatese activity- enzyme that synthetise estrogen.
Estrogen-like activity has been described for metals (aluminium, uranium, lead), polycyclic aromatic hydrocarbons (PAH), heterocyclic amines, some pesticides, dioxins, some antibiotics, arsenic.
Multiple functions of agents

Heavy metals bind to estrogen receptors

DNA damage via

1. interference with cellular redox regulation causing oxidative DNA damage or trigger signaling cascade stimulating cell growth;
Multiple functions of agents

binds to estrogen receptors

DNA damage via

1. interference with cellular redox regulation causing oxidative DNA damage or trigger signaling cascade stimulating cell growth;
2. inhibition of major DNA repair systems resulting in genomic instability and accumulation of critical mutations;
Multiple functions of agents

binds to estrogen receptors

DNA damage via

1. interference with cellular redox regulation causing oxidative DNA damage or trigger signaling cascade stimulating cell growth;
2. inhibition of major DNA repair systems resulting in genomic instability and accumulation of critical mutations;
3. deregulation of cell proliferation by induction of signaling pathways or inactivation of growth controls such as tumor suppressor genes

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Multiple functions of agents

binds to estrogen receptors

DNA damage via

1. interference with cellular redox regulation causing oxidative DNA damage or trigger signaling cascade stimulating cell growth;
2. inhibition of major DNA repair systems resulting in genomic instability and accumulation of critical mutations;
3. deregulation of cell proliferation by induction of signaling pathways or inactivation of growth controls such as tumor suppressor genes
   - aneugen
   - hypomethylation

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Aromatase inhibitors

• cotinine
• fungicides
• antiepileptic drugs
Foetus is transplacentally exposed to:

- food additives

- radiochemically contaminated water and air

- sometimes drugs (antiepileptics, antibiotics, antimycotics)
• Foetus's detoxification capacity buffers some of genotoxic agents.

• Target organs for xenobiotics (and genotoxic effects) may be different in mother and the foetus due to a specific stage of developmental physiology and enzyme distribution.

• This in turn may lead to different levels of clastogenic or aneugenic metabolites or DNA methylation after exposure to the same xenobiotic in the mother and the foetus.
Transplancental genotoxicity

Genome damage is result of complex interaction between:

- maternal and fetal metabolism (hepatic and extrahepatic)
- fetal developmental stage and pregnancy-related bioaccumulation
- detoxification capacity of the fetus and mother (including endometrium)

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• Epidemiological studies on parental exposure and cancer risk in children are limited.

• The correlation between cancer in children and occupational exposure of parents was found in a case of parental occupational exposure to pesticides, paternal exposure to motor vehicle-related occupations, chemical and petroleum industry, glue, nuclear plant (more than 100 mSv) and paint, maternal exposure to solvents, professional cooking,
the miscarriage incidence in women occupationally exposed to radioisotopes (iodine, chromium, thallium, technetium, thorium) in hospitals is significantly increased in comparison with women occupationally exposed to X rays showing that health effect of radioisotopes or mixtures (contaminated due to technology of production by lead, tin, nickel)

Elements in application for diagnostics is not limited on their radioactivity but also may act as heavy metals or xenoestrogens
Correlation of miscarriage rate and follow up by genetical toxicology (Fučić et al., 2008)

<table>
<thead>
<tr>
<th>Cytogenetic Endpoints</th>
<th>Study Groups</th>
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<tbody>
<tr>
<td></td>
<td>Referents (No. = 36)</td>
<td>X-ray exposed (No. = 170)</td>
<td>R-isotope exposed (No. = 61)</td>
<td></td>
</tr>
<tr>
<td>Chromosome aberrations</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
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<tr>
<td>Chromatide</td>
<td>1.71 ± 1.08</td>
<td>1.97 ± 1.41</td>
<td>1.98 ± 1.47</td>
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<tr>
<td>Chromosome</td>
<td>0.15 ± 0.33</td>
<td>0.51 ± 0.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.63 ± 0.99&lt;sup&gt;a&lt;/sup&gt;</td>
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</tr>
<tr>
<td>Acentric</td>
<td>0.36 ± 0.47</td>
<td>0.60 ± 0.90</td>
<td>0.61 ± 1.09</td>
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<tr>
<td>Dicentrics</td>
<td>0.0</td>
<td>0.12 ± 0.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.16 ± 0.36&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

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<table>
<thead>
<tr>
<th>Health Endpoints</th>
<th>Female population</th>
<th>Study Groups</th>
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<tbody>
<tr>
<td></td>
<td>a</td>
<td>X-ray exposed</td>
<td>R-isotope exposed</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(No. = 170)</td>
<td>(No. = 61)</td>
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<tr>
<td>No. of pregnancies</td>
<td>465712</td>
<td>187</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>No. of miscarriages</td>
<td>35165</td>
<td>19</td>
<td>33</td>
<td></td>
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<tr>
<td>Miscarriage Rate (%)</td>
<td>7.6</td>
<td>10.2</td>
<td>29.5</td>
<td></td>
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</tbody>
</table>

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• Diethylstilbestrol /Fucic et al. submitted

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Micronucleus frequency in three and twelve-week-old mice exposed to 0.05 µg/kg in males and females.

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Micronucleus frequency in three and twelve-week-old mice exposed to 0.5 µg/kg in males and females.

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Micronucleus frequency in three and twelve-week-old mice exposed to 5 µg/kg in males and females.
Transplacental genotoxicity

Mothers & newborns

- Antiepileptic drugs
- (aromatese inhibitors or estrogen coupling agents)
- 50 couples over the last 2 years
  - Sampling of the mother and the 3-day-old newborn

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Fluconazole (aromatase inhibitor)

(Fucic et al, 2008)
• 5-nitrofurantoin
• Metabolism liver, cecum and colon
Comparison of Genomic Damage Caused by 5-Nitrofurantoin in Young and Adult Mice using the in vivo Micronucleus Assay

Fucic et al, EMS 2005

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Ionizing radiation
Indoor radon

• Chronic exposure to indoor radon is associated with childhood cancers; correlation coefficient for all cancers 0.78 (p < 0.01), leukemia 0.61 (p< 0.02), brain and spinal 0.62 (p< 0.02), osteosarcoma 0.56 (p< 0.05), melanoma 0.56 (p<0.05)
WATER

- use of bottled water, beverages popular in children and adolescents.

- the total body concentration of radionuclides and equivalent doses to red bone marrow are age dependent and it is higher in children, especially in infants for $^{226}$Ra, $^{210}$Pb, $^{228}$Th, $^{210}$Po

- $^{226}$Ra follows the metabolic pathway of calcium

- the increased effective dose from mineral water might be even seven times higher in infants and teens than recommended by WHO (100 μSv). This may be related to a specific hormonal activity of testosterone and estrogen.

- highest absorption of $^{226}$Ra is in newborns and in children in age between 13 and 17 years of age

- high water intake in newborns and children

- un-nursed infants up to 1 year of age may receive doses up to 0.2 mSv/y if their diet is exclusively prepared with mineral water with elevated radon concentrations.
Epidemiology

- Significant correlation between cancer incidence in children and residence within 0-5 km from nuclear plants in Germany, RR 1.22

- Occupational exposure of fathers working in nuclear plant, receiving a total preconceptual dose of 100 mSv or more is associated with increased rate of childhood leukemia and lymphoma RR 6.4 (1.57-26.3)

- For childhood lymphatic leukemia 1.55 (CI 1.00-2.41) incidence and mortality 2.74 (CI 1.42-5.27)
## Techa River

*(M. Bauchinger, 1998)*

<table>
<thead>
<tr>
<th>Year of birth</th>
<th>No. of persons studied</th>
<th>Mean year of birth+SD</th>
<th>No. of cells scored</th>
<th><em>F_G</em> per 1000 cells+SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1914-1936</td>
<td>14</td>
<td>1927±6</td>
<td>6510</td>
<td>9.7 ± 2.3</td>
</tr>
<tr>
<td>1937-1949</td>
<td>14</td>
<td>1944 ± 5</td>
<td>6905</td>
<td>22.0 ± 4.3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>28</strong></td>
<td><strong>1936 ± 10</strong></td>
<td><strong>13415</strong></td>
<td><strong>16.0 ± 2.7</strong></td>
</tr>
</tbody>
</table>

* F_G value (full genomic equivalent)
Evacuated children from Chernobyl and control children from St Petersburg
(Lyubimova, NE, Vorobtsova, IE, Rad Biol Radioecol, 2008)

- Lymphocytes irradiated with 1.5Gy (gamma)

<table>
<thead>
<tr>
<th>Age</th>
<th>Control</th>
<th>Exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-10 (7.2)</td>
<td>38.6±1.1</td>
<td>47.3 ±0.8</td>
</tr>
<tr>
<td>11-20 (12.7)</td>
<td>40.4 ±1.1</td>
<td>49.4 ±1.3</td>
</tr>
<tr>
<td>21-30 (26.2)</td>
<td>42.4 ±1.2</td>
<td>36.2 ±0.9</td>
</tr>
<tr>
<td>31-40 (36.3)</td>
<td>39.8 ±1.0</td>
<td>36.5 ±0.6</td>
</tr>
</tbody>
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Synthetic biology

Complexity

- Xenobiotic levels
- Time/development
- Biomonitoring
- Semantic networks
Oncology in front of genotoxicology and environmental health.

Oncology departments have data on metabolism, environmental and occupational background which can be easily incorporated in already existing clinical questionnaires.
Large datasets produced by molecular biology (functional genomics) encourage scientists to take systems biology as a tool to synthesise interpretations of the interaction between complex radiochemical (living and occupational) environment and the organisms.
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- genotoxicologists
- endocrinologists
- oncologists
- environmental health experts
Free data flow

Artificial intelligence

System freedom
(change its own structure)

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Scientists are those who are fearful if they cannot control and understand

No more random and by chance

Chris Busby

Institute for Medical Research and Occupational Health, Croatia
Disease Gene Network

Kwang-II Goh et al. 2007
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