Phytochemicals and diet in health & disease: epigenetic friends or foe?

Sitges 2011

Wim Vanden Berghe
Outline

1. Nutrition & cancer-inflammation: the good and the bad
2. Can anti-inflammatory effects of phytochemicals explain their potential anti-cancer effects?
3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects
1. Nutrition & cancer-inflammation: the bad news

**Increased cancer risk in developed regions**

Adults in North America, Alaska, Australia and parts of Europe have a higher risk of getting cancer before age 65.

The probability of developing a cancer before age 65, 2002

- no data
- 5.0%–7.4%
- 7.5%–9.9%
- 10.0%–12.4%
- 12.5%–14.9%
- 15.0% and above

**SOURCE:** American Cancer Society
1. Nutrition & cancer-inflammatory: the bad news

<table>
<thead>
<tr>
<th>Disease / condition</th>
<th>Influence of diet, %</th>
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</thead>
<tbody>
<tr>
<td>Cardiovascular</td>
<td>&gt; 30</td>
</tr>
<tr>
<td>Cancers</td>
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<tr>
<td>Constipation</td>
<td>&gt; 70</td>
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<tr>
<td>Obesity</td>
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<td>Diabetes type 2</td>
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<tr>
<td>Dental caries</td>
<td>&gt; 30</td>
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Table 1. Estimated influence of diet on specific diseases
### 1. Nutrition & cancer: the good news?

Differences in cancer incidence related to diet preference?

<table>
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<tr>
<th>Cancer</th>
<th>USA Cases</th>
<th>USA Deaths</th>
<th>India Cases</th>
<th>India Deaths</th>
<th>Japan Cases</th>
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<td>102</td>
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<td>Nasopharynx</td>
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<td>2</td>
<td>4</td>
<td>3</td>
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<td>2</td>
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<tr>
<td>Other Pharynx</td>
<td>19</td>
<td>9</td>
<td>57</td>
<td>42</td>
<td>10</td>
<td>7</td>
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<td>Oesophagus</td>
<td>31</td>
<td>31</td>
<td>63</td>
<td>59</td>
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<tr>
<td>Stomach</td>
<td>56</td>
<td>34</td>
<td>43</td>
<td>39</td>
<td>489</td>
<td>225</td>
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<td>Colon/Rectum</td>
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<td>139</td>
<td>40</td>
<td>26</td>
<td>342</td>
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<td>Liver</td>
<td>30</td>
<td>31</td>
<td>17</td>
<td>16</td>
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<td>Pancreas</td>
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<td>68</td>
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<td>11</td>
<td>76</td>
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<td>11</td>
<td>35</td>
<td>22</td>
<td>17</td>
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<td>Lung</td>
<td>463</td>
<td>402</td>
<td>55</td>
<td>51</td>
<td>262</td>
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<td>Melanoma of skin</td>
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<td>21</td>
<td>3</td>
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<td>Breast</td>
<td>914</td>
<td>212</td>
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<td>Cervix uteri</td>
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<td>33</td>
<td>307</td>
<td>174</td>
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<td>Corpus uteri</td>
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<td>20</td>
<td>17</td>
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<td>45</td>
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<td>Ovary etc.</td>
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<td>62</td>
<td>49</td>
<td>29</td>
<td>66</td>
<td>37</td>
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<td>46</td>
<td>28</td>
<td>111</td>
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<td>Testis</td>
<td>40</td>
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<td>6</td>
<td>3</td>
<td>13</td>
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<td>Bladder</td>
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<td>28</td>
<td>20</td>
<td>16</td>
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<td>17</td>
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<td>Kidney etc.</td>
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<td>6</td>
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<td>19</td>
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<td>Brain, nervous system</td>
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<td>Multiple myeloma</td>
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<td>26</td>
<td>8</td>
<td>6</td>
<td>16</td>
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<tr>
<td>Leukemia</td>
<td>80</td>
<td>54</td>
<td>26</td>
<td>20</td>
<td>48</td>
<td>34</td>
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<tr>
<td>All sites but skin</td>
<td>3223</td>
<td>1391</td>
<td>1017</td>
<td>688</td>
<td>2230</td>
<td>1213</td>
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</tbody>
</table>

*Showing cases were after standardized with world standard population, called World Standardized incidence or mortality rate. It is also expressed per million.* - J. Ferlay, et al. GLOBOCAN 2000. URL: http://www-dep.iarc.fr/globocan/globocan.htm
2. Can anti-inflammatory effects of phytochemicals explain their potential anti-cancer effects

NFkB promotes 7 cancer hallmarks and its activity is increased in most tumors

- NFkB, AP1
- MMP, IL6, COX2
- NFkB, AP1, IL6
- COX2
- IL8
- MMP
- IL6
- NFkB, p53
- NFkB, AP1
- Cyclin D, c-myc, telomerase
- NFkB, Stat3
- A1/Bfl
- A20
- cIAP
- Mdr/Pgp
- BclXl
- NFkB, p21
- VEGF, IL6
- Insensitivity to growth inhibitors
- Tissue invasion & metastasis
- Evasion of apoptosis
- Self-sufficiency in growth signals
- Limitless replicative potential
- An inflammatory microenvironment
2. Can anti-inflammatory effects of phytochemicals explain their potential anti-cancer effects

Chronic inflammation promotes tumor initiation-progression, angiogenesis and metastasis
2. Can anti-inflammatory effects of phytochemicals explain their potential anti-cancer effects

Hypothesis: Various dietary phytochemicals lower basal inflammatory state
2. Can anti-inflammatory effects of phytochemicals explain their potential anti-cancer effects

Plant derived NFkB inhibitors attenuate cancer hallmarks in vitro in concentration range >10µM ...

Natural products can inhibit NFkB activity at various levels:
1. Nutrition & cancer: the good and the bad
2. Can anti-inflammatory effects of phytochemicals explain their potential anti-cancer effects?

**Problem:** various anti-inflammatory mechanisms are demonstrated at supraphysiological concentrations >10µM

3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects
4. Conclusions
Outline

1. Nutrition & cancer: the good and the bad
2. Can anti-inflammatory effects of phytochemicals explain their potential anti-cancer effects?
3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects?

Epigenetics definition: **Heritable** changes in phenotype and/or gene expression that occur without a change in DNA sequence

**Heritable:** transgenerational, mitotic stable, perpetuation of gene activity in absence of the original signal
3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects

![The epigenome diagram](chart.png)

**An Epigenetic Switch Involving NF-κB, Lin28, Let-7 MicroRNA, and IL6 Links Inflammation to Cell Transformation**

Dimitrios Iliopoulos, Heather A. Hirsch, and Kevin Struhl
Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA

MicroRNA-Dependent Regulation of DNA Methyltransferase-1 and Tumor Suppressor Gene Expression by Interleukin-6 in Human Malignant Cholangiocytes

Chiara Braconi, Nianyuan Huang, and Tushar Patel
3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects
3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects

**Epigenetic dogma of cancer**

**Global hypomethylation**

**Hypermethylation of tumor suppressor genes**

*Figure 1. Epigenetic Alterations in Tumor Progression.*
A multistage model of carcinogenesis in skin is shown. In conjunction with phenotypic cellular changes and the accumulation of genetic defects, there is a progressive loss of total DNA methylation content, an increased frequency of hypermethylated CpG islands, and an increased histone-modification imbalance in the development of the disease. H-ras denotes Harvey-ras oncogene, and 5mC 5-methylcytosine.

*Figure 2. DNA methylation pattern in normal and cancer cells.* CpG islands are protected from methylation in normal cells. CpG sites away from transcription start sites and in repetitive elements are typically methylated. The situation gets reversed in cancer resulting in focal hypermethylation and global hypomethylation. CpG islands flanking start sites of some genes may become methylated. Intragenic CpG sites and repeats are become unmethylated. Green lollipops show unmethylated, red lollipops methylated CpG sites.
Maternal Supplements
With Genistein
Zinc methionine
Betaine choline,
Folate B$_{12}$

Lower risk of cancer, diabetes, obesity, CVD and prolonged life

3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects - key experiments

Agouti model: R. Waterland and R. Jirtle

Germ cells carry the epigenetic benefits of grandmother’s diet

Craig A. Cooney*
Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR 72205
Agouti mouse model: Soy diet induces transgenerational changes in DNA methylation which protect 2 unexposed generations to obesity & cancer.
3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects - key experiments

Timing of dietary exposure maybe critical to achieve chemopreventive effects

**Figure 2** | Environmental sensitivity of the epigenome throughout life. The top row indicates normal reprogramming of the epigenome during gametogenesis, fertilization and development. The bottom row indicates the environmental cues that affect the epigenome and have late-life consequences, and the stages of life at which they act. Sensitivity of the epigenome to the environment (represented by shading of the arrow) is likely to decrease during life as growth slows. Abbreviation: IVF, *in vitro* fertilization.
3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects - key experiments

Early Life Exposure to Famine and Colorectal Cancer Risk: A Role for Epigenetic Mechanisms

Laura A. E. Hughes¹, Piet A. van den Brandt¹, Adriaan P. de Bruïne², Kim A. D. Wouters², Sarah Hulsmans², Angela Spiertz³, R. Alexandra Goldbohm³, Anton F. P. M. de Goeij², James G. Herman⁴, Matty P. Weijenberg¹, Manon van Engeland²*

¹ Department of Epidemiology, GROW School for Oncology and Developmental Biology, Maastricht University Medical Center, Maastricht, The Netherlands, ² Department of Pathology, GROW School for Oncology and Developmental Biology, Maastricht University Medical Center, Maastricht, The Netherlands, ³ Department of Prevention and Health, TNO Quality of Life, Leiden, The Netherlands, ⁴ Sidney Kimmel Comprehensive Cancer Center, John Hopkins University School of Medicine, Baltimore, Maryland, United States of America

Gluckman et al. Genome Medicine 2010, 2:14
http://genomemedicine.com/content/2/2/14

COMMENTARY

Developmental origins of health and disease: reducing the burden of chronic disease in the next generation

Peter D Gluckman¹,², Mark A Hanson³ and Murray D Mitchell⁴
Concentration of chemicals including man-made and natural in umbilical cords/cord blood from newborns in Japan

Mori C.,
76: 361-368 (2001)

3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects?
3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects?

<table>
<thead>
<tr>
<th>FOOD</th>
<th>CHEMICAL</th>
<th>EPIGENETIC ROLE</th>
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<tbody>
<tr>
<td>Sesame Seeds</td>
<td>Methionine</td>
<td>Methylates DNA (gene silencing)</td>
</tr>
<tr>
<td>Nuts</td>
<td>Folic Acid</td>
<td>Methylates DNA (gene silencing)</td>
</tr>
<tr>
<td>Sunflower Seeds</td>
<td>Folic Acid</td>
<td>Methylates DNA (gene silencing)</td>
</tr>
<tr>
<td>Peppers</td>
<td>Methionine</td>
<td>Methylates DNA (gene silencing)</td>
</tr>
<tr>
<td>Spinach and Other Leafy Vegetables</td>
<td>Methionine and Folic Acid</td>
<td>Methylates DNA (gene silencing)</td>
</tr>
<tr>
<td>Broccoli</td>
<td>Sulphoraphane</td>
<td>Acetylates Histones (activating genes)</td>
</tr>
<tr>
<td>Other Vegetables</td>
<td>Vitamin B6</td>
<td>Methylates DNA (gene silencing)</td>
</tr>
<tr>
<td>Garlic</td>
<td>Diallylsulphide (DADS)</td>
<td>Acetylates Histones (activating genes)</td>
</tr>
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<td>Soy or Soy Products</td>
<td>Choline, Genistein</td>
<td>Methylates DNA (gene silencing)</td>
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<td>Beef</td>
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<td>Choline</td>
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<td>Chicken</td>
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<td>Liver</td>
<td>Folic Acid</td>
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</tr>
<tr>
<td>Egg Yolk</td>
<td>Choline</td>
<td>Methylates DNA (gene silencing)</td>
</tr>
</tbody>
</table>

Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development

Dana C. Dolinoy††, Dale Huang*, and Randy L. Jirtle*†††

*Department of Radiation Oncology and †University Program in Genetics and Genomics, Duke University, Durham, NC 27710; and ‡Integrated Toxicology and Environmental Health Program, Duke University, Durham, NC 27708

Edited by R. Michael Roberts, University of Missouri, Columbia, MO, and approved June 25, 2007 (received for review April 23, 2007)
3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects? A case study

Withaferin A inhibits inflammatory gene expression in MDA-MB231 breast cancer cells in [nM] range

Widespread ethnopharmacological use of *Withania Somnifera* / *Ashwagandha* (Ayurvedic) extracts for cancer chemotherapy and anti-inflammatory use
3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects? A case study

Withaferin A blocks breast cancer metastasis in vivo in nM range

Thaiparambil et al. 2011, Int. J. Cancer, DOI: 10.1002/ijc.25938
3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects? A case study

Differential IL6 gene expression in weak versus strong metastatic breast cancer cell types

**A**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>MCF7</th>
<th>MDA-MB231</th>
<th>MDA-MB436</th>
<th>T47D</th>
<th>ZR75</th>
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<tbody>
<tr>
<td><strong>Relative mRNA IL6/HKG</strong></td>
<td>![Bar Chart]</td>
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</tr>
</tbody>
</table>

**ERα-positive, low metastatic, weak IL6 gene expression levels**
MCF7, T47D, ZR75

**ERα-negative, strong metastatic, aggressive**

**Strong IL6 gene expression levels**
MDA-MB231, MDA-MB468

**B**

**MCF7**

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<thead>
<tr>
<th>Time</th>
<th>IL6</th>
<th>GAPDH</th>
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<tr>
<td>12</td>
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</table>

**MDA-MB231**

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<td>12</td>
<td>![Image]</td>
<td>![Image]</td>
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</tbody>
</table>
3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects? A case study

Phytochemical effects on histone modifier enzymes selectively affects specific NFKB target genes
3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects? A case study

Oncogene chromatin opening involved in breast cancer metastasis

Experimental Design: Nucleosome Position

Indirect End-Labeling Technique

DNase

Metastatic Non-metastatic

\[ ^{32}\text{P}-\text{probe} \]

Unique RE

MCB 2009 Ndlovu et al.
3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects? A case study

Phytochemicals also regulate chromatin dynamics of inflammatory cells (DC)

Dijsselbloem
J. Immunol. 2007
3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects? A case study

Reverse oncogenic gene expression by increased DNA methylation via dietary compounds?
3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects? A case study

MBD2 seq based profiling of the cancer methylome/immune cell methylome

**Principle MBD2 sequencing:**

DNA methylation Signature is cell specific

Relative methylation MDA/MCF7

Methylation changes WA/untreated

DNA methylation

Breast

Myeloma
3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects? A case study

Complexity of methylation dynamics
3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects? A case study

**Dietary effects on methylation signature in breast cancer**

IPA analysis

DNA methylation profiling reveals a predominant immune component in breast cancers, Dedeurwaerder et al. In press 2011
3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects? A case study

Possible mechanisms of diet dependent DNA (de)methylation dynamics

Depletion methyl donors
Decreased CpG Me

Inhibition Dnmt
Decreased CpG Me

Genotoxicity – oxidative damage
Decreased CpG Me
3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects? A case study

Is epigenetic drift a consequence of aerobic life?

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The Redox Basis of Epigenetic Modifications: From Mechanisms to Functional Consequences

ANTIOXIDANTS & REDOX SIGNALING
Volume 15, Number 2, 2011
© Mary Ann Liebert, Inc.
DOI: 10.1089/ars.2010.3492

Anthony R. Cyr and Frederick E. Domann
3. Can epigenetic effects of phytochemicals explain their potential anti-cancer effects? A case study

**Xenohormesis: interspecies epigenetic stress response**

![Diagram of metabolic pathways and xenohormesis]

**Xenohormesis: Sensing the Chemical Cues of Other Species**

Konrad T. Howitz* and David A. Sinclair*

*Cell 133, May 2, 2008 ©2008 Elsevier Inc.*
Conclusions

Epigenetic reprogramming of the cancer-immunomethylome may contribute in chemopreventive effects of early life exposure to physiological concentrations of dietary phytochemicals

Future:
Study methylation dynamics (turnover) in function of age (pregnant, neonatal, puberty, old) or single/repeated exposure

Compare methylation dynamics following exposure to dietary polyphenols versus bisphenols: rationale for beneficial/detrimental effects in cancer?

Study crosstalk metabolic pathways vs. dynamics DNA methylation/hydroxymethylation, histone methylation
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Karen Heyninck
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Wim Van Criekinge
Tim Demeyer

University Antwerp
Ajay Palagani
Katarzyna Szarc vel Szic

University Brussels ULB
Carine Van Lint
Francois Fuks

University Montpellier
Inserm U540
Dany Chalbos
If they ask you anything you don’t know, just say it's due to epiqenetics.