“Nutrigenomics and Personalized Medicine”

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“Nutrigenomics and Personalized Medicine”

Learning Objectives

- Define a bioactive food component
- Understand intraindividual variations in response
- Define interaction between cancer and food components such as selenium
- Define how diet can shift transcriptomic expression
- Explain long term effects of soy intake as it relates to cancer
Unprecedented opportunities exist for the expanded use of foods and components to achieve genetic potential, increase productivity and reduce the risk of disease, including cancer.
Hippocrates Proclaimed almost 2500 years ago:

Let thy food be thy medicine and thy medicine be thy food.
Public Health Approach
There Are Always A Few Skeptics!

“Snow White was poisoned by an apple, Jack found a giant in his beanstalk, and look what happened to Alice when she ate the mushroom! And you wonder why I won’t eat fruit and vegetables!”
The Causes of Cancer

Diet 35%
Other Factors 35%
Tobacco 30%
Other 35%
In 2006 about 720K new cases in men and about 670K cases in women. Death attributed to cancer in 2006 will be about 290K and 270K, respectively.

Projections of Cancer Cases between 2000 to 2050 by Age

Source: SEER program, NCI and population projections from US Census Bureau
While Diet Linked to Cancer Risk: Much Confusion Exist About What to Eat

"Low fat diets don’t work. I eat fish every day and my butt still drags on the ground!"
### Relative Risk of Breast Cancer: Influence of Soy

#### Asian

<table>
<thead>
<tr>
<th>Study</th>
<th>Intervention</th>
<th>Menopausal Status</th>
<th>Relative Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lee '92 (total soy protein)</td>
<td>p &lt; 0.001 Premenopausal</td>
<td>NS Postmenopausal</td>
<td>2.6</td>
</tr>
<tr>
<td>Hirose '95 (bean curd, miso)</td>
<td>NS Premenopausal</td>
<td>NS Postmenopausal</td>
<td>1.3</td>
</tr>
<tr>
<td>Yuan '95 (tofu, soymilk)</td>
<td>NS Premenopausal</td>
<td>NS Postmenopausal</td>
<td>1.0</td>
</tr>
<tr>
<td>Wu '96 (tofu)</td>
<td>p &lt; 0.01 Premenopausal</td>
<td>p &lt; 0.05 Postmenopausal</td>
<td>0.44 – 0.79 Shanghai, Tianjin</td>
</tr>
</tbody>
</table>

#### Key '99 (soy)

- Tofu
- Miso

#### Zheng '99 (urinary isoflavonoids)

#### Dai '01 (soy)

- NS All Breast Cancer
- S Just ER+/PR+

#### Wu '02 (soy)

#### Yamamoto '03 (isoflavonoid consumption)

- Premenopausal
- Postmenopausal

#### Western

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<th>Menopausal Status</th>
<th>Relative Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wu '04 (soy)</td>
<td></td>
<td></td>
<td>1.33</td>
</tr>
</tbody>
</table>

Ingram '97 (urinary isoflavones)

- NS Diadzein
- p = 0.009 Equol

Witte '97 (soy)

den Tonkelaar '01 (urinary phytoestrogens)

Horn-Ross '01 (phytoestrogen intake)

Keinan-Boker '02 (food content)

Linseisen '04 (isoflavone intake)

- daidzein and genistein

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### Notes

- The data above represents the relative risk of breast cancer influenced by soy consumption.
- The relative risks are given for different menopausal statuses and interventions.
- The significance levels (p-values) are indicated for each study.
The “Oomics” May Explain Variation in Response

Nutrigenetics
Nutritional Epigenetics
Nutritional Transcriptomics
Proteomics
Metabolomics
Phenotype
Protein
Metabolite
RNA
DNA

Bioactive Food Component

“Nutritional Preemption”
Concept that bioactive food components can be introduced at points of initiation & progression for pathway leading to an unhealthy or lethal phenotype
Biological Determinants of the Response to Bioactive Food Components (3 Biomarkers)

- Dietary Exposure
- Absorbed Dose (?)
- Inactive Metabolite
- Biologically Effective Dose
- Susceptibility Factors
- Molecular Target
- Early Biologic Effect
- Health Benefits + and -
- Altered Structure/Function
Credentialing of nutrients and molecular targets is likely the future?
Credentialing is defined as “omic” changes that bring about a phenotypic change.
Biomarkers of Quantity and Intended Use

Response

Toxicity
Cell Cycle Inhibition
Apoptosis
Differentiation
Immune Enhancement
Carcinogen Metabolism
Antioxidant

Exposure

Suspect Functional Foods With Health Benefits

- Soy
- Tomatoes
- Spinach
- Broccoli
- Garlic
- Nuts
- Salmon
- Oats
- Blueberries
- Green tea
- Red wine

Modified Time Magazine: January 21, 2002
Physiological exposures

Absorption
Metabolism
Distribution
Excretion

24 hour record
Food frequency questionnaire
Food diary
Individual Consumption of All Tomatoes and Tomato Products and Serum Lycopene Levels (EPIC Cross-sectional Study in 3000 subjects)

\[ \text{Corr} = 0.23 \]
Not All Tissues Equally Influenced by Bioactive Food Components

Question Remains About True Site of Action of Functional Foods and Health Benefits
Approximately 25% of the mice develop a single tumor

.....this is a new mouse model of sporadic colon cancer, which represents the vast majority of human colon cancer

Vitamin D and Cancer: Current Dilemmas/Future Needs
May 7–8, 2007
Vitamin D, Calcium and Cancer


The Genetic Revolution Is Providing New Insights into a Number of Health Issues Including the Role of Diet in Cancer Prevention

Genotype of Metabolic Enzymes Modifies the Benefit of Tamoxifen

- Cytochrome P4502D6 is involved in the metabolism of tamoxifen
- Among individuals homozygous for CYP2D6*1 genotype, there was no benefit of tamoxifen
- Patients containing at least one CYP2D6*4 allele had better survival (RR=0.28) when randomized to tamoxifen

Wegman and Wingren Breast Cancer Res. 7:R284-R290, 2005
All Individuals Will Not Respond Identically!
Myeloperoxidase Polymorphism (G463A) and Higher Fruit/Vegetable Intake

Risk not related to polymorphism when intakes was low. Low and high consumption based on median values of control group: fruit and vegetable, 29 svg/wk


Premenopausal- greatest response to increased fruits and vegetables
Genetic Information May Assist in Identifying Those Who Must Assure Adequate Intakes


Dietary Calcium

OR for Colon Cancer

VDR Genotype

< 388 mg/day

>388 mg/day

P for trend=0.004

Genetic Information May Help Identify Those At Risk and to Formulate Appropriate Interventions

Not Knowing Genomics Can Cause Misinterpretation

Yang et al, Cancer Res. 61, 565, 2001
PPAR Delta Genotype, Fish Consumption and Colon Cancer

(384 cases and 403 polyp-free controls 789C-->T)

Siezen et al., Carcinogenesis 26:229-457, 2005

P <0.01 for interaction

Odds Ratio

CC, p<0.06
CT +TT, p<0.02

Tertile Fish Intake (g/d)

Siezen et al., Carcinogenesis 26:229-457, 2005
Breast Cancer Prevention Trial (BCPT)
Events Among Participants

Is It Logical To Assume That Foods Or their Components Result in No Risk??
Our results do not support the hypothesis that high folate intake reduces breast cancer risk; instead, they suggest that a high intake, generally attributable to supplemental folic acid, may increase the risk in postmenopausal women.

Folic Acid Supplement Intervention May Also Increase Colon Cancer Risk

603 association of polymorphisms and disease

166 studies with at least 3 population

Only 6 reproducible (>75 % studies)

Genetic Testing Promises vs Reality!

• Commercial Nutrition-Gene Test
  – Genelex Sciona 19 genes including MTHFR $395
  – Gene Care CVD nutritional genetic test (South Africa) MTHFR (Hcyst), apoA1 (HDL) +9 others $400

• Exceeding complex area since about 30,000 Genes, 8-10 Million SNPs

***Opportunities available for SBIR and STTR for Kits (Screens) for Predicting Risk
Diets Are Complex Since Contain More than 25,000 Bioactive Components

- **Essential Nutrients** - Ca, Zn, Se, Folate, Vitamins D, C, E
- **Non-Essential Phytochemicals** - Carotenoids, Flavonoids (Anthocyanin), Isothiocyanates, Allyl Sulfur, Zoochemicals - Conjugated linoleic acid, n-3 fatty acids
- **Fungochemicals** - Several compounds in mushrooms
- **Bacteriochemical** – Those formed from food fermentations and those resulting from intestinal flora

Complexity of diet is Illustrated by the Vast Number of Foods and Components which can modify the cancer process
Bacterial Formed Equol May Account for Part of the Anticancer Properties from Soy in Asian-Americans


demonstrated that isoflavone treatment in subjects who have the capacity to produce equol differentially affects gene expression as compared with nonproducers.

Expression of a large number of genes was altered by isoflavone treatment, including induction of genes associated with cyclic adenosine 3′,5′-monophosphate (cAMP) signaling and cell differentiation and decreased expression of genes associated with cyclin-dependent kinase activity and cell division.

In general, isoflavones had a stronger effect on some putative estrogen-responsive genes in equol producers than in nonproducers.
Transgenic and Knockout Models
Key to Identifying Sites of Action of Food Components

Prostate Tumors in *Lady* mice.

Antioxidants= vitamin E, selenium and lycopene

Venkateswaran et al., Cancer Research 64: 5891-5896, 2004
Response Also Evident in Humans
But Tissue Specific Response

Design Phase III trial with 1,312 skin cancer subjects

<table>
<thead>
<tr>
<th></th>
<th>Selenium</th>
<th>Placebo</th>
<th>RR</th>
<th>97% CI</th>
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</thead>
<tbody>
<tr>
<td><strong>Skin Cancer</strong></td>
<td></td>
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<tr>
<td>Squamous cell</td>
<td>218</td>
<td>190</td>
<td>1.14</td>
<td>0.93-1.39</td>
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<tr>
<td>Basal cell</td>
<td>377</td>
<td>350</td>
<td>1.10</td>
<td>0.95-1.28</td>
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<tr>
<td><strong>Cancer (- Skin)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>17</td>
<td>31</td>
<td>0.54</td>
<td>0.30-0.98</td>
</tr>
<tr>
<td>Prostate</td>
<td>13</td>
<td>35</td>
<td>0.37</td>
<td>0.18-0.71</td>
</tr>
<tr>
<td>Colorectal</td>
<td>8</td>
<td>19</td>
<td>0.42</td>
<td>0.18-0.95</td>
</tr>
<tr>
<td>All Sites (- Skin)</td>
<td>77</td>
<td>119</td>
<td>0.63</td>
<td>0.47-0.85</td>
</tr>
</tbody>
</table>

Se (200 μg) as Brewers Yeast vs. Placebo, follow-up 6.4 yrs

Also Clear That All People Do Not Respond Identically

MY GENOME MADE ME DO IT!
Epigenetic Regulation of Cancer

Factors
- Environmental
- Diet
- Hormonal
- Genetic

Epigenetics regulates:
- Cell Cycle Control
- DNA Damage
- Apoptosis
- Invasion
- X-Chromosome Inactivation
- Imprinting
- Aging

Diet, Epigenetic Events, and Cancer Prevention.


DNA Methyl-transferases
- DNMT1
- DNMT3A
- DNMT3B

siRNA

Global Hypomethylation

Site Specific Hypermethylation

Histone Changes
- Acetylation
- Changes
When to Intervene??

Maternal Supplements with zinc, methionine betaine, choline, folate, B$_{12}$

Or

Genistein

ltr Hypomethylated

Yellow Mouse

High risk cancer, diabetes, obesity & reduced lifespan

ltr Hypermethylated

Agouti Mouse

Lower risk of cancer, diabetes, obesity and prolonged life

Oxidation of either a single guanine to 8-oxoguanine or of a single 5mC to 5-hydroxymethylcytosine significantly inhibits binding of the methyl-CpG binding proteins to the oligonucleotide duplex, reducing the binding affinity by at least an order of magnitude.

Oxidative damage to DNA could therefore result in heritable, epigenetic changes in chromatin organization.

Reactivation of Genes by Dietary Components

Trichostatin = TSA 5-aza-dCyd = DAC

Histones Can Be Regulated by Isothiocyanates, Allyl Sulfur, Genistein and Butyrate

- Acetylated histones H3 and H4 associated with P21 and Bax promoters
- Transcription of P21 and Bax mRNA
- p21 and Bax protein levels increased
- Cell cycle arrest
- Caspase activation
- APOPTOSIS

Influence of diet on other histone modification process remains unknown
Effect of 68 g of BroccoSprouts on Histone Deacetylase Activity in Humans

Dietary Intervention Can Cause Shifts in Transcriptomic Expression

"It would never work, Nichole; I'm in the experimental group and you're in the control."
Transcriptomic Markers Are Providing Clues About Molecular Targets for Specific Food Components

Selenium in prostate cancer cells:
- Androgen Signaling
- Proliferation/Cell cycle
- Detoxification
- Immune/stress
- Apoptosis
- Transcription
- Signal Transduction
- Cell Shape

Differentially expressed genes in human blood leukocytes after consumption of the high-protein (HP) breakfast, the high-carbohydrate (HC)

Combinations May Influence The Quantity Needed For A Response?

Garlic, fish, broccoli

Active Intermediate (radical??)

HS  SH

keap-1  nrf2

Agent

S  S

keap-1

Cytoplasm

nrf2

Nucleus

ARE

“antioxidant responsive element”

Increased GST, QR
Recent News Release suggests: Herceptin is a Novel Pioneering Drug for Personalized Medicine Approach Based on Pharmacogenomics to block Her2-neu expression.

Evidence Has Existed for Years that: EGCG from Green Tea, Oleic Acid from Olive Oil, and n-3 fatty acids from Fish Oil and Apigenin from parsley, thyme, and peppermint can significantly influence HER2neu expression!

So why are we not being more proactive in showcasing dietary effects?
Herceptin and Dietary fish oil increased the latency time to mammary gland tumor development in the HER-2 transgenic mice.


Yee LD et al J Nutr 135: 983-8, 2005
Food/Food Components (n-3/Butyrate/ Herbs/Spices,)

- Inflammatory Stimuli (chemicals, ROS, bacteria, viruses)
- Polymorphisms (Pro- and Anti Inflammatory Genes)

Inflammatory Response

Disease

Morbidity

Mortality
K14-HPV16 Transgenic Mouse

No Estrogen
Normal Diet

Estrogen
Normal Diet

Estrogen
Diet + I3C

Proliferation Assay: PCNA by immunohistochemistry

Auborn et al, Personal Communication
Elevated thiobarbituric acid reactive substances, conjugated dienes and lowered activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and reduced glutathione in the liver, heart, kidney, intestine and aorta observed in rats fed the high fat diet was minimized or eliminated by supplementation with black pepper or the active principle of black pepper, piperine.

DNA

Nutrigenetics
Nutritional Epigenetics
Nutritional Transcriptomics

Proteomics
Metabolomics

Bioactive Food Component

RNA

Protein
Metabolite

Needs & Insults
Cellular Process(es)
Phenotype
Proteomics and Metabolomic Technologies and the Response to Bioactive Food Components

Mitchell et al. (2005) Proteomics 5: 2238
Identified 2 peaks that reflect with 76% accuracy broccoli intake

Various proteins modified by quercetin

Exercise and quantitative proteomics

Vitamin A dependent proteins.

Primary Effect of Allyl Sulfur is to Block Cell Division by Changing Phosphorylation

DADS

Inactive Kinase

P14 P15 P161

Active Kinase

P161

We Must Be Careful About Messages to Public

One Size Does Not Fit All! Genomic Background Will Help Identify Responders to Foods and Components

More is Not Always Better. Energy Intakes and Expenditures Are Linked to Health!
Body Mass Index and Cancer Mortality Women

Source: Calle et al NEJM 2003; 348: 1625-38

20% of cancer deaths in women
Could Obesity Be Reflecting Some Other Metabolic Effect??

Snijder, et. al. (2005) J Clin Endo & Metab 90:4119–4123
Timing of Dietary Change
Also
Important!!
Teenage (13-15 y) Soy Intake & Adult Breast Cancer Risk

(Shanghai: 1459 cases, 1556 cont)

Results: pre/post, x age 47. Other legumes not protective.

May Take Time to Observe A Response!

Women’s Intervention Nutrition Study (WINS)

**RELAPSE EVENTS**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Control</th>
<th>HR, 95% CI</th>
<th><em>p</em>-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>96/975</td>
<td>181/1462</td>
<td>0.76, 0.60-0.98</td>
<td>0.034</td>
</tr>
</tbody>
</table>

* From adjusted Cox proportional hazards model including: stratification factors, ER status, tumor size, and surgery (mastectomy/lumpectomy), p value = 0.067 by unadjusted log rank test

Linxian Nutrition Intervention Trial
Esophageal cancer mortality by factor D (N=1515)

Factor D= Selenium, β-carotene, vitamin E

Taylor, P. et al., Gastroenterology 2005 (abstract)
When I knew all of life’s answers, they changed all the questions!
The Future
Transform Medicine from Curative to Preemptive

Predictive ↔ Personalized ↔ Preemptive

Participatory
Could Defining a Nutritional Phenotype Be the Future for Reducing Risk and Enhancing Therapy?
Be Creative in Whatever You Do
Preclinical or Clinical
Develop Partnership: Build on Strengths
Unprecedented Analytical Issues Surround Defining: Which Dietary Components Are Most Important In What Amounts and When!!

Who will Respond Favorable and Who will be Placed at Risk
Review

- Why is there variation in response to specific food components?
  1. Different genetic metabolism
  2. Different absorption profiles
  3. Different excretion time profiles
  4. All of the above

- Which of the following terms applies to the food components effect on DNA?
  1. Nutrigenetics
  2. Nutritional Epigenetics

- Of the 3 Vitamin D receptor genotypes (TT, Tt & tt) which is the most effected by increased caffeine intake?
  1. TT
  2. Tt
  3. tt

- What dietary changes mimic the effects of Herceptin in HER2 neu expression?
  1. EGCG from green tea
  2. N-3 fatty acids from Fish Oil
  3. Apigenin from parsley, thyme and peppermint
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