Genomic Analysis of Metabolic Pathway of Phase 1 and Phase 2 Detoxifying / Antioxidant Defense Enzymes Modulated by Dietary Phytochemicals

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Rutgers, The State University of New Jersey

COST-Meeting in Vienna, Austria
Molecular and Physiological Effects of Bioactive Food Compounds
Vienna, October 11-16, 2006
Healthy Compounds Isolated from Fruits, Vegetables and Tea

- **Tea polyphenols – Green & black Tea**
- **Curcumin – Turmeric curry**
- **Isothiocyanates – broccoli, Brussels Sprout**

Cancer, Cardiovascular Dis., CNS, Inflammatory Dis., Metabolic Dis.
Dietary Phytochemicals Are Overall Health Beneficial (Normal Cells)

- For normal non-high risk individuals, these phytochemicals could benefit (at “lower dietary doses”) almost like multi-vitamins? This could also include many Natural Products?

- Could promote or enhance the health & protective effects against “oxidative injuries” not just for Cancer, but also cardiovascular, CNS, inflammatory and metabolic diseases?

- Via Modulation of different signaling pathways and gene expression in different tissues (overall Health Beneficial).
Dietary Phytochemicals are Effective Cancer Preventive Agents (Pre-Cancerous Cells)

- ITCs (SFN or PEITC) are effective against intestinal, prostate and skin tumor models
- Combination of half of the doses of PEITC and curcumin are as effective in prostate cancer (A Pharmacological Approach)
- These compounds can modulate different signaling pathways in different tumor tissues origin (targeting heterogeneity of cancers in patients, earlier lesions?)
- Early diagnosis of high risk patients?
Progression and Prevention of Cancer

HEALTHY CELL

OLTIPRAZ VITAMINE CALCIUM

GENETIC MUTATIONS THAT CAN LEAD TO CANCER

DAMAGED CELL (PRECANCEROUS)

CANCER CELLS

DIFFERENTIATION OF CELLS

PROGRAMMED DEATH OF ALTERED CELLS (APOPTOSIS)

(4-HPR)

TAMOXIFEN NSAIDS DFMO FINASTERIDE

PROCESSSES THAT LEAD TO EXCESSIVE PROLIFERATION OF GENETICALLY DAMAGED CELLS

Peter Greenwald, Scientific American, 1996
Many Chemopreventive Compounds Can Induce both Blocking and Suppressing Effects

Cellular Signals

Genes Level

Blocking Effects
Normal Cells

Suppressing Effects
Cancer Cells
Blocking Agents

- Blocking activation of potential carcinogens (P450)
- Induce Phase II Detoxifying and Cellular Defense Enzymes – more critical?
Dietary Detoxifying Enzyme Inducers - Blockers

A. Chemopreventive agents
   - Blocking agents
   - Suppressing agents

Normal cells → Initiation → Mutant cells

Promotion → Benign tumors → Progression → Malignant tumors

DNA damage

B. Dietary detoxifying enzyme inducers functioning as blocking agents

Carcinogens / activated procarcinogens by CYPs → Inactivation by detoxifying enzymes → Less reactive & faster elimination

Examples of dietary chemopreventive compounds functioning as Phase II detoxifying enzyme inducers

<table>
<thead>
<tr>
<th>Class of Chemical</th>
<th>Representatives</th>
<th>Source</th>
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<tbody>
<tr>
<td>Phenols</td>
<td>Ferulic acid</td>
<td>Rice, fruits</td>
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<tr>
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<tr>
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<td>EGCG</td>
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<td>Brassinin</td>
<td>Cruciferous vegetables</td>
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<td>Indole-3-carbinol</td>
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<td>Kahweol</td>
<td>Green coffee bean</td>
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<td>Coumarins and lactones</td>
<td>Coumarin</td>
<td>Leguminosae species</td>
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<tr>
<td></td>
<td>Auraptene</td>
<td>Citrus fruits</td>
</tr>
<tr>
<td>Inorganic</td>
<td>Selenium</td>
<td>Meat, wheat, dairy or fish</td>
</tr>
</tbody>
</table>

Eg., Many Phytochemicals Including Anti-Oxidants and Isothiocyanates Induce cellular defense - Phase II Drug Metabolizing Enzymes (DME)

- Glutathione S - Transferases (GST)
- Quinone reductases (QR)
Compounds Isolated From Broccoli, Grapes, and Green Tea

- How these compounds transduce or translate their signals from outside the cell to inside the cell where our genes are
- Affect changes in genes express level
Xenobiotics-induced Chemical Stress – SUL/PEITC, EGCG

**Normal Cells**
- Survival Response
  - Ras
  - Raf
  - MEK1/2
  - ERK
  - NFκB•IκB
- AP-1, NFκB Survival Genes: c-Jun, c-Fos, cdks
- ARE: Defense Genes - GST, QR, MT, HO

**Chem/Oxidative Stress GSH / Protein Thiols**
- Ca++
- PKC
- MEKK1-3
- TAK1
- MEK4/7
- MEK3/6
- JNK
- p38

**Mitochondria**
- Bcl-2
- Caspases 3 (CPP32), 6, 7

**Tumor Cells**
- Apoptotic Response
- ARE: Defense Genes - GST, QR, MT, HO
- Survival Response
- Ras
- Raf
- MEK1/2
- ERK
- NFκB•IκB
- PKC
- MEKK1-3
- TAK1
- MEK4/7
- MEK3/6
- JNK
- p38

**Apoptosis**
Many Phytochemicals are Inducers of Phase II Genes

Phenolic Antioxidants
Phytochemicals Isothiocyanates

Increase Phase II Detoxifying Enzymes
GST, QR, UGT

Lee Wattenberg, 1970s
Phytochemicals are Inducers of Phase II Genes & Are Electrophiles

Phenolic Antioxidants (EGCG)
Phytochemicals Isothiocyanates

Transcription Activation
ARE (antioxidant response element) or EpRE (Electrophile response element)

Increase Phase II/Defense Enzymes
GST, QR,, UGT, gGCS, HO-1

ARE – Cecil Pickett
EpRE – Violet Daniel & Paul Talalay
1.6 kb 5’-flanking region of the \textit{rat GST-ya} gene. GRE, glucocorticoid-responsive element; XRE, xenobiotic-responsive element; HNF, hepatocyte nuclear factor; \textit{ARE}, \textit{antioxidant-responsive element}.

The phenolic antioxidant BHA and its metabolite tBHQ can strongly activate this reporter gene (more appropriate to be called “Oxidative Stress Response Element” – activated by ROS-H2O2 & Cd, Ar)
Isothiocyanates – broccoli, cauliflower, water cress

PITC

N=C=S

PMITC

N=C=S

PEITC

N=C=S

Sulforaphane

PBITC

N=C=S

PHITC

N=C=S
Isothiocyanates – broccoli, cauliflower, water cress

- PITC
- PMITC
- PEITC
- Sulforaphane
- TOXIC

N=C=S

- PBITC
- PHITC
Induction of ARE-mediated Reporter Gene By Various Isothiocyanates

Pharm. Res. 13: 1043, 1996
Green tea polyphenols

EC

ECG

EGCG

Arch. Pharm. Res. 2000
Induction of Luciferase by Green Tea Compounds
Garlic Organosulfur - Diallyl Disulfides (DAS)

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34, 2004
Effects of PEITC (water cress) on MAPK (JNK)
PEITC Activates JNK1 Activity in HeLa and HT1080 Sarcoma Cells

<table>
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<tr>
<th>HeLa Cells</th>
<th>PEITC (µM)</th>
<th>0</th>
<th>1</th>
<th>5</th>
<th>10</th>
<th>30</th>
<th>50</th>
<th>100</th>
<th>300</th>
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</table>

Cancer Res 56: 2954, 1996
**Effects of Oxidants/Reductants on System**

- Activation of MAPK (JNK) by PEITC requires protein sulfhydryl groups and/or GSH

Cancer Res 56: 2954, 1996
Figure 3 E – Sulforaphane Treated Rat Livers

Phosphorylation of MAPKs after 50 µmol SUL/rat

Hu et al., JPET, July 2004
PK in the Rats: SUL (50 umole) Plasma Conc’n Peaked 20 uM (TR 1-20 uM), and t1/2 = 2.3 hr

JPET, July, 2004
MAPK Cascade in Phase 2 Enzymes Induction

Phase 2 Enzyme Inducers
BHA, tBHQ, PEITC, SUL

Ras → Raf → MEK1/2 → ERK

Raf → MEKK1/ASK1 → TAK1

MEKK1/ASK1 → JNK → p38

SEK (MKK4/7) → MKK3/6

MAPKKK → MAPKK → MAPK

Aim 1 MAPK Pathways

Aim 2 MAPK Transcription

Aim 3 MAPK Phosphorylation

ARE-Mediated Phase 2 Gene NQO1

Kong, 1995
YW Kan, PNAS, 1994
Nrf2 was cloned using AP-1 site probe to screen a lambda gt11 cDNA expression library from K562 cells
Upregulation of Nrf2 - ARE transcriptional activity by the Ras- and Raf-dependent pathways

JBC, 275: 2000
Regulation of Nrf2 Transcription Activity by MAPK Pathways

Chemical Stress

- Ras
  - Raf-1
  - MEKK1
  - ?

- MEK1/2
  - MKK7
  - MKK4
  - MKK6
  - MKK3

- ERK1/2
- JNK1/2
- p38

- ARE

Phase 2 Enzymes: GST, QR, HO-1, γGCS, Fer-L, TR

JBC, 275: 2000
PEITC Activates Nrf2, ARE and MAPK in Human Prostate PC-3 cells

Molecular Cancer Ther. Aug 2006
PEITC Releases Nrf2-GFP from Keap1-DsRED

**EGFP-Nrf2 + DsRed-Keap1**

DAPI  Keap1  Nrf2  Merged

**EGFP-Nrf2 + DsRed-Keap1 + PEITC**

DAPI  Keap1  Nrf2  Merged

Molecular Cancer Ther.  Aug 2006
JNK Releases Nrf2-GFP from Keap1-DsRED

MKK4+JNK1+EGFP-Nrf2+DsRed-Keap1

DAPI  Keap1  Nrf2  p-JNK

Molecular Cancer Ther. Aug 2006
ERK Releases Nrf2-GFP from Keap1-DsRED

MEK1+ERK2+EGFP-Nrf2+DsRed-Keap1

DAPI Keap1 Nrf2 p-ERK

DAPI DAPI DAPI DAPI
Keap1 Keap1 Nrf2 Keap1
Nrf2 pERK p-ERK Nrf2

Molecular Cancer Ther. Aug 2006
BHA Releases Nrf2-GFP from Keap1-DsRED

30 min

1 h

2 h

4 h  Mol. Carcinog.  In press

<table>
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<tr>
<th>DAPI</th>
<th>Nrf2</th>
<th>Keap-1</th>
<th>Merge</th>
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</table>

30 min

1 h

2 h

4 h  Mol. Carcinog.  In press
Nrf2 – NES (Nuclear Export Signal), Redox Insensitive & Overlapping with bZIP Dimerization Domain

Nrf2

EGFP-Nrf2zip

EGFP-NES-GAL4DBD

EGFP-GAL4DBD

GST-Nrf2zip

JBC, 2005
Nrf2 – NES (Nuclear Export Signal), Redox Insensitive NES abrogated by mutation of the leucine residues

A - GFP-Gal4DBD
B – DAPI - DNA
C - Superimposed

D- NES-1-L-Mut
E – NES-2-L-Mut
F – NES-3-L-Mut

JBC, 2005
A Redox Sensitive NES in TAD

Fig. 1 Li et al.

JBC, Sept, 2006
Nrf2-NES-TA Expels Nrf2 out of the Nucleus under basal condition

A

EGFP-NES_{TA}    PI    Merged

EGFP-NES_{INT}    PI    Merged
NES-TA of Nrf2 Responds to Oxidants such as DEM, H2O2, SUL, tBHQ

EGFP-NES$_{TA}$

+DMSO  +DEM  +H$_2$O$_2$  +tBHQ

+GSH  +Sul  +GSH & Sul

EGFP-NES$_{TA}$ C183A

JBC, Sept 2006
NES-TA Does NOT bind to Keap1

A

FRET assay

CFP-NES\textsubscript{TA} + YFP-Keap1

CFP-Nrf2\textsubscript{NT} + YFP-Keap1

YFP Channel | CFP Channel | FRET Channel

B

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fret_assay.png}
\caption{FRET assay showing the interaction between CFP-NES\textsubscript{TA}, CFP-Nrf2\textsubscript{NT}, and YFP-Keap1.}
\end{figure}

C

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fret_analysis.png}
\caption{FRET analysis showing the interaction between CFP, CFP-Nrf2\textsubscript{NT}, ECFP-NES\textsubscript{TA}, EYFP-Keap1, and Anti-Keap1.}
\end{figure}

JBC, Sept 2006
Balancing between Nuclear Import (NLS) and Export (NES) – Oxidants will modify NES – Retained in Nucleus

JBC, Sept 2006
Nrf2 binds to p160 SRC and transactivate Nrf2/ARE transcription

JBMB, 2006
Chemical Signal

Keap1 - Nrf2

Kinases

Phase II Gene Expression

POL II Complex

Keap1

Nrf2

SH

Chemical Signal

Kinases

Phase II Gene Expression

POL II Complex

Keap1

Nrf2

SH

Chemical Signal

Kinases
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<tr>
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<th>Vehicle</th>
<th>Preventive Compounds</th>
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<tr>
<td>Wild Type</td>
<td>wt/ V</td>
<td>wt/ Rx</td>
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<tr>
<td>Nrf-2^{−/−}</td>
<td>ko/ V</td>
<td>ko/ Rx</td>
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</table>
Nrf2 Knock-out Mice

- More sensitive to **BaP-induced** and blunted oltipraz-protection (Ramos-Gomez et al., PNAS, 2001) & sulforaphane-protection (Fahey et al., PNAS, 2002) against gastric carcinogenesis in Nrf2 KO mice

- More prone to **liver damage by acetaminophen** (Enomoto et al., Tox. Sci. 2001)

- More prone to **Pulmonary injury by butylated hydroxytoluene** (Chan et al., PNAS, 1999) and hyperoxic injury (Cho et al., AJRCMB, 2002)

- Increased **DNA adducts** in lungs after exposure to diesel exhaust (Aoki et al., TAP, 2001)

- Are these effects mainly because of induction of ARE-mediated cellular protective genes? What are they?
Nrf2 KO mice studies

Affy 45 K Probes Chips
Vehicle Control
(50% PEG 400, PO)

C57BL/6J
4 Mice

Sacrificed at 3h

SIT
Liver

RNA (Pooled)

DNA

C57BL/6J/Nrf2(-/-)
4 Mice

SIT
Liver

RNA (Pooled)

DNA

TM Treatment
(2mg/kg, PO)

C57BL/6J
4 Mice

Sacrificed at 3h

SIT
Liver

RNA (Pooled)

DNA

C57BL/6J/Nrf2(-/-)
4 Mice

Sacrificed at 3h

SIT
Liver

RNA (Pooled)

DNA

Nair and Kong, Tox. Let, 2006 (accepted)
Design of Experiment and Data Flow

Genes regulated over two-fold by treatment in Nrf2(+/-) mice

Genes regulated over two-fold by treatment in Nrf2(-/-) mice

Nrf2-dependent genes
Spearman correlation of microarray and real-time PCR data

$r^2 = 0.96$
Nrf2-dependent regulation of Phase I genes by chemopreventive agents in mice liver

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>PEITC</th>
<th>SFN</th>
<th>EGCG</th>
<th>Curcumin</th>
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<tbody>
<tr>
<td></td>
<td>3h</td>
<td>12h</td>
<td>3h</td>
<td>12h</td>
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<tr>
<td>Phase I CYP450 Genes</td>
<td></td>
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<tr>
<td>CYP450, 2c37</td>
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<tr>
<td>CYP450, 2j9</td>
<td>2.1</td>
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<td>CYP450, 4a10</td>
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<td>CYP450, 4a14</td>
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Nrf2-dependent regulation of Phase II genes by chemopreventive agents in mice liver

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<tr>
<th>Gene Name</th>
<th>PEITC 3h</th>
<th>PEITC 12h</th>
<th>SFN 3h</th>
<th>SFN 12h</th>
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<th>EGCG 12h</th>
<th>Curcumin 3h</th>
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<td>GCL, catalytic subunit</td>
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<td>GCL, catalytic subunit</td>
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Nrf2-dependent regulation of antioxidant genes by chemopreventive agents in mice liver

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<th>PEITC 12h</th>
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<th>SFN 12h</th>
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<td>Ferritin light chain 1</td>
<td></td>
<td>2.2</td>
<td>2.2</td>
<td>2.2</td>
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<tr>
<td>Glutathione peroxidase 6</td>
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<tr>
<td>Glutathione reductase 1</td>
<td></td>
<td>2.2</td>
<td></td>
<td>2.2</td>
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<tr>
<td><strong>Heme oxygenase 1</strong></td>
<td></td>
<td><strong>12.2</strong></td>
<td></td>
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<td><strong>4.9</strong></td>
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<tr>
<td>Thioredoxin reductase 1</td>
<td></td>
<td>2.6</td>
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<td>Thioredoxin reductase 2</td>
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<td>Thioredoxin-like</td>
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</table>

### Nrf2-dependent regulation of ATP-binding cassette family (ABC) transporter genes by chemopreventive agents in mice liver

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>PEITC</th>
<th>SFN</th>
<th>EGCG</th>
<th>Curcumin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3h</td>
<td>12h</td>
<td>3h</td>
<td>12h</td>
</tr>
<tr>
<td>Transporter Genes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABCB1a, MDR1, Pgp</td>
<td>3.8</td>
<td>2.8</td>
<td>2.6</td>
<td>2.2</td>
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<tr>
<td>ABCB1b, MDR1, Pgp</td>
<td>5.0</td>
<td>6.1</td>
<td>6.7</td>
<td>7.1</td>
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<tr>
<td>ABCB3, TAP2, ABC18</td>
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<td>2.8</td>
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<tr>
<td>ABCC2, MRP2, ABC30</td>
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<tr>
<td>ABCC3, MRP3</td>
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<tr>
<td>ABCC5, MRP5</td>
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<td>ABCD2, ALDR, ABC39</td>
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<tr>
<td>ABCD3, ABC43</td>
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<td>ABCF3</td>
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</table>

**Nrf2-dependent regulation of solute carrier family (SLC) transporter genes by chemopreventive agents in mice liver**

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>PEITC</th>
<th>SFN</th>
<th>EGCG</th>
<th>Curcumin</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>3h</td>
<td>12h</td>
<td>3h</td>
<td>12h</td>
</tr>
<tr>
<td>Transporter Genes</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>SLC9A8, NHE8 (Na+/H+)</td>
<td>2.2</td>
<td>3.7</td>
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<tr>
<td>SLC22A3, OCT3</td>
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<td></td>
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</tr>
<tr>
<td>SLC22A5, OCTN2</td>
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<td>2.4</td>
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<td>2.2</td>
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<tr>
<td>SLC22A6, OAT1</td>
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</tr>
<tr>
<td>SLC22A13</td>
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<tr>
<td>SLC26A1, SAT1 (sulfate)</td>
<td>3.0</td>
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<td>2.0</td>
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<tr>
<td>SLCO3A1, OATP-D</td>
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<td>3.6</td>
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<td>9.4</td>
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<td>SLCO6D1, OATP6D1</td>
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<td>4.7</td>
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</table>

How about in the small intestine (SIT)?
EGCG and Curcumin regulated Nrf2-dependent Phase II genes in mice SIT

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>EGCG 3h</th>
<th>EGCG 12h</th>
<th>Curcumin 3h</th>
<th>Curcumin 12h</th>
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<tbody>
<tr>
<td>Phase II Genes</td>
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<td>GST, alpha 2 (Yc2)</td>
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<td>GST, alpha 3</td>
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<tr>
<td>GST, alpha 4</td>
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<td>3.8</td>
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<tr>
<td>GST, mu 1</td>
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<td>11.7</td>
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<tr>
<td>GST, mu 3</td>
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<td>5.9</td>
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<tr>
<td>GST, mu 4</td>
<td>5.4</td>
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<tr>
<td>GST, mu 6</td>
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<tr>
<td>UGT, 2b5</td>
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<td>14.1</td>
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</table>

EGCG and Curcumin regulated Nrf2-dependent antioxidant genes in mice SIT

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>EGCG 3h</th>
<th>EGCG 12h</th>
<th>Curcumin 3h</th>
<th>Curcumin 12h</th>
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<tbody>
<tr>
<td>Antioxidant Genes</td>
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<td>Carbonyl reductase 3</td>
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<tr>
<td>Epoxide hydrolase 1</td>
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<tr>
<td>Ferritin light chain 1</td>
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<td>2.3</td>
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</tr>
<tr>
<td>GCLC</td>
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</tr>
<tr>
<td>Glutathione reductase 1</td>
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<tr>
<td>Heme oxygenase 1</td>
<td>4.2</td>
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<td>Thioredoxin reductase 1</td>
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<tr>
<td>Thioredoxin reductase 3</td>
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</table>

Summary of Nrf2 KO/Affy Chips

Detoxification genes
CYP450 genes
Transporter genes
Apoptosis
Kinase, Phosphatase
SFN, Curcumin, EGCG, PEITC
Ubiquitination
Transcription factor
DNA repair
Cell cycle control
Cell adhesion

Summary of Nrf2 KO mice

- **Consequences** of regulation of some of these genes could lead to:
- Affecting **other drugs** absorption, metabolism and PK, when given together
- But most importantly, regulation of these genes could also play important roles in **diseases prevention** by these compounds such as **cancer**, CNS, cardiovascular, inflammatory and metabolic diseases.
Nrf2 +/- Knock-out Mice

Sulforaphane inhibits skin tumorigenesis in C57BL/6 mice associated with Nrf2 gene

2-Stage Carcinogenesis Model - Mice were treated with 200 nmol DMBA, and one week later, 8 nmol TPA were applied twice a week for 25 weeks.

Changjiang Xu, Ph.D.

Collaborators:
MT Huang, Ph.D.
Allan Conney, Ph.D.

Cancer Res. Sept 2006
Fig 1. Skin tumor incidence. Mice were pretreated with 100 nmol SFN for 14 d, after that, 200 nmol DMBA was applied on the second day, one week later, 8 nmol TPA were applied twice a week for 25 weeks.
# In Skin Tumor Samples – Lost of Nrf2 and HO-1 Expression

<table>
<thead>
<tr>
<th></th>
<th>Nrf2(+/+)</th>
<th>Nrf2(-/-)</th>
<th>Nrf2(+/+)</th>
<th>Nrf2(-/-)</th>
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</thead>
<tbody>
<tr>
<td>Nrf2</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>HO-1</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Actin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Non-tumor samples  Tumor samples

*Cancer Res. Sept 2006*
In Skin Tumor Samples – Over-Expression of Cell Growth & Inflammatory Genes

- c-Myc
- Bcl-2
- Cyclin D1
- β-catenin
- VEGF
- Cox-2
- Bax
- p-AKT
- Actin

Non-tumor samples  Tumor samples

Cancer Res. Sept 2006
ITCs Inhibit IKK, NF-KB & related genes expression in human prostate PC-3 cells

Phospho-IkBα(Ser32)

β-actin

SFN  -  20 μM  30 μM  -  -
PEITC - - - 5 μM  7.5 μM

Phospho-IKKβ(Ser181)
Phospho-IKKα(Ser180)

β-actin

SFN  -  20 μM  30 μM  -  7.5 μM
PEITC - - - 5 μM  μM

VEGF
Cyclin D1
Bcl-XL

β-actin

SFN  -  20 μM  30 μM  -  -
PEITC - - - 5 μM  7.5 μM

Oncogene June 2005
**PEITC/Curcumin Inhibit AKT**

<table>
<thead>
<tr>
<th>Curcumin (μM)</th>
<th>-</th>
<th>25</th>
<th>35</th>
<th>-</th>
<th>-</th>
<th>25</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEITC (μM)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>10</td>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>

- p-PDK1
- p-Akt (S473)
- p-Akt (T308)
- AKT
- p-IkBα
- Actin

*Carcinogenesis 2005*
PEITC/Curcumin Inhibit EGFR-PI3K

Carcinogenesis 2005
PEITC/Curcumin Inhibit IKK/NF-KB and PI3K/AKT/mTOR

Carcinogenesis 2005
Combined Inhibitory Effects of Curcumin and Phenethyl isothiocyanate (PEITC) on the Growth of Human PC-3 Prostate Xenografts in Immunodeficient Mice

Tin Oo KHor, Ph.D.
Avantika Golpalkrisna, M.S.
Young-Sam Keum, M.S.
Jung-Hwan Kim, M.S.
Bandaru Reddy, Ph.D.
Xi Zheng, Ph.D.
Allan Conney, Ph.D.
A.-N. Tony Kong, Ph.D.

Cancer Res.
Jan 15, 2006
PEITC & Curcumin alone or in combination
Inhibit human PC-3 xenografts in nude mice

I.P. injection every MWF for 28 days; starting 1 day before PC-3 cells injection:

PEITC 5 umole
Curcumin 6 umole
PEITC 2.5 umole + cur 3 umole

Cancer Res.
Jan 15, 2006
PEITC & Curcumin – Increased apoptosis

Control PEITC Curcumin PEITC + Curcumin

Percentage of Apoptotic Cells (%)

Control  PEITC  Curcumin  PEITC + Curcumin

Percentage of Proliferative Cells (%)

Control  PEITC  Curcumin  PEITC + Curcumin

Cancer Res.
Jan 15, 2006
Chemopreventive Effects of Sulforaphane on the Growth of Adenomas Polyps in APCmin Mice

Rong Hu, Ph.D.
Tin Oo KHor, Ph.D.
Guoxiang Shen
Woo-Sik Jeong
Vidya Hebbar
Chi Chen
Changjiang Xu
Bandaru Reddy
Kiran Chada
A.-N. Tony Kong, Ph.D.
The representative slide showing the H and E staining of normal and adenomatous (arrow) mouse intestine from APC min mice. A: 200X; B: 20X
Sulforaphane: 3-weeks study in APC min mice (11 weeks old)

- after 3 weeks, the animals were sacrificed:

- Control – AIN 76 A diet – 44.4 adenomas

- Sulforaphane 300 ppm – 32.6 adenomas
  (26% Reduction in numbers)

- Sulforaphane 600 ppm – 27.5 adenomas
  (38% reduction in numbers)

- Also reduction in Sizes of adenomas
Concentrations of SFN and SFN-GSH in Plasma and in Tissues of APC min

<table>
<thead>
<tr>
<th>SFN content in diet (ppm)</th>
<th>Plasma levels (nM)</th>
<th>Small intestine levels (nmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SFN</td>
<td>SFN-GSH</td>
</tr>
<tr>
<td>300</td>
<td>124±44</td>
<td>579±106</td>
</tr>
<tr>
<td>600</td>
<td>254±136</td>
<td>770±103</td>
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</table>

Total concentrations of SFN and SFN-GSH in plasma and in tissues of APC min = 3 – 10 µM

Carcinogenesis
Oct 2006
<table>
<thead>
<tr>
<th>Gene</th>
<th>1d</th>
<th>3d</th>
<th>5d</th>
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<tr>
<td>Metallothionein 1</td>
<td>1.83±0.29</td>
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<td>2.42±0.43</td>
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<tr>
<td>tumor necrosis factor receptor superfamily, member 7</td>
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<td>2.21±0.2</td>
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<tr>
<td>checkpoint suppressor 1</td>
<td>3.06±0.63</td>
<td>2.3±0.36</td>
<td>2.06±0.34</td>
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<tr>
<td>tumor necrosis factor (ligand) superfamily, member 11</td>
<td>1.93±0.48</td>
<td>3.47±0.29</td>
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<tr>
<td>COX-2 (prostaglandin-endoperoxide synthase 2)</td>
<td>0.45±0.16</td>
<td>0.53±0.24</td>
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<tr>
<td>cyclin D2</td>
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<td>0.49±0.07</td>
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<td>mitogen activated protein kinase kinase kinase 8</td>
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<td>0.2±0.03</td>
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<td>interleukin 6</td>
<td>0.25±0.02</td>
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</tbody>
</table>

Drug Disp Biopharm.
Sept 2006
Dietary Phytochemicals are Effective Cancer Preventive Agents (in cancer therapy?)

- ITCs (SFN or PEITC) are effective against intestinal, prostate and skin tumor models.
- Combination of half of the doses of PEITC and curcumin are as effective in prostate cancer (A Pharmacological Approach).
- These compounds can modulate different signaling pathways in different tumor tissues origin (targeting heterogeneity of cancers in patients, earlier lesions or prevent recurring cancer?).
- Early diagnosis of high risk patients?
Dietary Phytochemicals Are Overall Health Beneficial
(normal cells)

- For normal non-high risk individuals, these phytochemicals could benefit (at “lower dietary doses”) almost like multi-vitamins? This could also include Tocotrienols?
- Could promote or enhance the health & protective effects against “oxidative injuries” not just for Cancer, but also cardiovascular, CNS, inflammatory and metabolic diseases?
- Via Modulation of different signaling pathways and gene expression in different tissues
Chemopreventive agents block carcinogenesis in NORMAL Cells via Nrf2/ARE-mediated genes expression.
Chemopreventive agents block carcinogenesis in TUMOR Cells by inhibiting cell growth signals and promote apoptosis.
Acknowledgements

- Guoxiang Shen, M.S.
- Wenge Li, Ph.D.
- Youngsam Keum, M.S.
- Sujit Nair, M.S.
- Wen Lin, M.S.
- Avantika Gopalkrishnan, M.S.
- Jung-Hwan Kim, M.S.
- Khor Tin Oo, Ph.D.
- Jin-Liern Hong, B.S.
- Siwang Yu, Ph.D.
- Xiaoling Yuan, Ph.D.
- Auemduan Prawan, Ph.D.
- Ki Han Kim, Ph.D.

Collaborators

- Jefferson Chan (UC Irvine)
- Allan H. Conney (Rutgers)
- M.T. Huang (Rutgers)
- Bandaru Reddy (Rutgers)
- Celine Gelinas (UMDNJ)
- Eileen White (Rutgers)
- CS Yang (Rutgers)
- KV Chin (Rutgers)
- Ronald Hart (Rutgers)
- Thomas Rushmore (Merck)
- Cecil Pickett (Schering)
- Tse-Hua Tan (Baylor)
- William Fahl (Wisconsin)
- Michael Weber (Virginia)
- Roger J. Davis (HHMI, U. Massachusetts)

Supported by NIH Grants CA-073674, CA-092515, CA-094828, CA-118947 and Funding from Glaxo Chair

- Rong Yu
- Sandhya V. Mandlekar
- Adedigbo Fasanmade
- Juah-Lin Duh
- Shu-yan Wang
- George A. Matwyshyn

- Zhiqiang Jiang
- Wei Lei
- Jie-jin Jiao
- Edward Owour, PhD.
- In-Wha Kim, Ph.D.
- Bok Ryang Kim, Ph.D.

- Vidya Hebar, Ph.D.
- Woo-Sik Jeong, Ph.D.
- Chi Chen, Ph.D.
- Rong Hu, Ph.D.
- Mohit Jain, Ph.D.
- Changjiang Xu, Ph.D.