Phase II Enzyme induction by phytochemicals: Perspective in Cancer Chemoprevention

Hassan Ahmad, Ph.D.
Professor of Biochemistry
Department of Chemistry
University of Texas-Pan American
Edinburg, TX
Where is UTPA?
Cancer Statistics 2013
2013 Estimated US Cancer cases

Estimated New Cancer Cases* in the US in 2013

<table>
<thead>
<tr>
<th>Cancer Site</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>28%</td>
<td>29%</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>14%</td>
<td>14%</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>9%</td>
<td>9%</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>6%</td>
<td>6%</td>
</tr>
<tr>
<td>Melanoma of skin</td>
<td>5%</td>
<td>6%</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>5%</td>
<td>4%</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>4%</td>
<td>4%</td>
</tr>
<tr>
<td>Oral cavity</td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td>Leukemia</td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td>All Other Sites</td>
<td>20%</td>
<td>19%</td>
</tr>
</tbody>
</table>

*Excludes basal cell and squamous cell skin cancers and in situ carcinoma except urinary bladder.

Source: American Cancer Society, 2013
# 2013 Estimated US Cancer Deaths

## Estimated Cancer Deaths in the US in 2013

<table>
<thead>
<tr>
<th>Cancer Site</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung &amp; bronchus</td>
<td>306,920</td>
<td>273,430</td>
</tr>
<tr>
<td>Prostate</td>
<td>14%</td>
<td>10%</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>9%</td>
<td>9%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>6%</td>
<td>7%</td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>5%</td>
<td>5%</td>
</tr>
<tr>
<td>Leukemia</td>
<td>4%</td>
<td>4%</td>
</tr>
<tr>
<td>Esophagus</td>
<td>4%</td>
<td>3%</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>4%</td>
<td>3%</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>3%</td>
<td>2%</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>3%</td>
<td>2%</td>
</tr>
<tr>
<td>All other sites</td>
<td>24%</td>
<td>25%</td>
</tr>
</tbody>
</table>

Source: American Cancer Society, 2013
## 10 Leading Causes of Death in US – 2010

<table>
<thead>
<tr>
<th>Rank</th>
<th>Cause of Death</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diseases of heart</td>
<td>597,689</td>
<td>24.2</td>
</tr>
<tr>
<td>2</td>
<td>Malignant Neoplasms (cancer)</td>
<td>574,743</td>
<td>23.3</td>
</tr>
<tr>
<td>3</td>
<td>Chronic lower respiratory diseases</td>
<td>138,080</td>
<td>5.6</td>
</tr>
<tr>
<td>4</td>
<td>Cerebrovascular diseases</td>
<td>129,476</td>
<td>5.2</td>
</tr>
<tr>
<td>5</td>
<td>Accidents (unintentional injuries)</td>
<td>120,859</td>
<td>4.9</td>
</tr>
<tr>
<td>6</td>
<td>Alzheimer disease</td>
<td>83,494</td>
<td>3.4</td>
</tr>
<tr>
<td>7</td>
<td>Diabetes mellitus</td>
<td>69,071</td>
<td>2.8</td>
</tr>
<tr>
<td>8</td>
<td>Nephritis and nephrosis</td>
<td>50,476</td>
<td>2.0</td>
</tr>
<tr>
<td>9</td>
<td>Influenza &amp; pneumonia</td>
<td>50,097</td>
<td>2.0</td>
</tr>
<tr>
<td>10</td>
<td>Intentional self-harm (suicide)</td>
<td>38,364</td>
<td>1.6</td>
</tr>
</tbody>
</table>


- **White**: Men 216.7, Women 150.8
- **African American**: Men 288.3, Women 174.6
- **Asian/Pacific Islander**: Men 132.6, Women 93.2
- **American Indian/Alaskan Native**: Men 184.9, Women 135.9
- **Hispanic†**: Men 146.4, Women 100.6

*Per 100,000, age-adjusted to the 2000 US standard population.
†Persons of Hispanic origin may be of any race.
Change in the US Death Rates* by Cause, 1950 & 2005

<table>
<thead>
<tr>
<th>Condition</th>
<th>1950</th>
<th>2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Diseases</td>
<td>586.8</td>
<td>211.1</td>
</tr>
<tr>
<td>Cerebrovascular Diseases</td>
<td>180.7</td>
<td>46.6</td>
</tr>
<tr>
<td>Influenza &amp; Pneumonia</td>
<td>48.1</td>
<td>20.3</td>
</tr>
<tr>
<td>Cancer</td>
<td>193.9</td>
<td>183.8</td>
</tr>
</tbody>
</table>

* Age-adjusted to 2000 US standard population.
Sources: 1950 Mortality Data - CDC/NCHS, NVSS, Mortality Revised.
Trends in Overweight* Prevalence (%), Adults 18 and Older, US, 1992-2010

*Body mass index ≥ 25.0 kg/m². Source: Behavioral Risk Factor Surveillance System, National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention.
Mechanism of carcinogen and detoxification

Pro carcinogens → Reactive electrophiles (active carcinogens) → DNA damage & adducts → DNA repair

- Normal DNA
- DNA damage
- Mutations and epigenetic changes
- Apoptosis

Detoxification products:

- GST, UGT
- NQO1
- Phase II enzymes

Nonelectrophilic metabolites:

- Cytochrome P450s
- Phase I enzymes

Cancer
Electrophiles Damage DNA, leading to cancer and other mutation-related health consequences.
PHASE I

P-450

PHASE I

OXIDATION

REDUCTION

HYDROLYSIS

PHASE II

ULTIMATE CARCINOGEN

PRO CARCINOGENS

GST

GSH

GSH-CONJUGATION PRODUCT

EXCRETION

DNA Damage
Strategies of Cancer Prevention

Inhibition of Phase I enzymes
Induction of Phase II enzymes
Induction of DNA repair enzymes
Glutathione

\[
\text{GLU} \quad \text{CYS} \quad \text{GLY}
\]

\[
+\text{NH}_3-\text{CH-C-N-CH-C-N-CH}_2-\text{COO}^-\quad \text{SH}
\]
GLUTATHIONE and DETOXIFICATION

Non enzymatic:
1. Major cellular nucleophile
2. Reducing agent
3. Free radical scavenger

Enzymatic:
1. GSH peroxidase I and II activities
2. Glutathione S-Transferase activity
GLUTATHIONE PEROXIDASE CATALYZED REACTION

2 GSH + RO-OH → ROH + GSSG

Peroxide

Respective alcohol

Toxic

Less toxic
Use and recycling of GSH

GSSG + ROH \rightarrow RO-OH \rightarrow ROH + 2 GSH

GSR \rightarrow HX \rightarrow RX \rightarrow GST

GR \rightarrow NADP \rightarrow NADPH \rightarrow G6PD \rightarrow G6P \rightarrow 6PGL

Mercapturic acid
GST-CATALYZED CONJUGATION OF CDNB TO GSH

GSH + Cl-NO₂-NO₂ → GS-NO₂-NO₂ + HCl

1-chloro-2,4-dinitrobenzene
Dinitrophenyl glutathione

INSOLUBLE: Toxic
SOLUBLE: Less toxic
GST-Mediated Conjugation of 9,10-epoxystearic acid with GSH

\[
\text{CH}_3-(\text{CH}_2)_7-\text{CH}-\text{CH}-(\text{CH}_2)_7-\text{C} = \text{O} + \text{GSH}
\]

9,10-Epoxystearic acid

\[
\text{CH}_3-(\text{CH}_2)_7-\text{CH}-\text{CH}-(\text{CH}_2)_7-\text{C} = \text{O} \quad \text{GST}\quad \text{pH 7.4}
\]

\[
\text{GSH-ESA}
\]

\[
\text{CH}_3-(\text{CH}_2)_7-\text{CH}-\text{CH}-(\text{CH}_2)_7-\text{C} = \text{O}
\]

\[
\text{OH} \quad \text{SG}
\]
Detoxification mechanism of DAD(P)H Quinone Oxidoreductase (QR)
FUNCTIONS OF GST

CATALYTIC FUNCTIONS:
1. Glutathione S-transferase activity
2. Glutathione Peroxidase II activity

NON CATALYTIC FUNCTIONS:
1. Reversible binding and transport of several organic compounds
2. Irreversible binding and transport of several electrophilic compounds including some carcinogen
CHARACTERISTICS OF GST

1. **High Concentrations in Tissues:** Rat liver 5%, human liver 3% of soluble proteins

2. **Multiple forms:** Present in most of the human tissues investigated so far.

3. **Multiple Functions:** Conjugate wide variety of xenobiotics to glutathione; Bind covalently and non-covalently to xenobiotics and Express glutathione peroxidase activity towards lipids and nucleic acid hydroperoxide
CHARACTERISTICS OF GST

4. **Induction:** Inducible by phenolic antioxidants and many xenobiotics and phytochemicals.

5. **Structure:** Most human isoenzymes arise from dimeric combination of several different subunits in the apparent Mr. range of 22K to 29K.

6. **Tissue Specific Expression:** Some tissues express only specific isoenzymes probably to satisfy the specific or unique needs of the tissue.
CLASSIFICATION OF GST

Major Classes

- Alpha Class
- Mu Class
- Pi Class
- Theta Class

Minor Classes

- Sigma Class
- Zeta Class
### Some of the known substrates of GST

<table>
<thead>
<tr>
<th><strong>Class</strong></th>
<th><strong>Example</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Halogennitrobenzenes</td>
<td>1,2,4-Trichloro-3-nitro benzene</td>
</tr>
<tr>
<td></td>
<td>1,2-dichloro-4-nitro benzene</td>
</tr>
<tr>
<td>Aralkyl halides</td>
<td>Benzyl chloride</td>
</tr>
<tr>
<td></td>
<td>4-bromobutylbenzene</td>
</tr>
<tr>
<td>Aralkyl esters</td>
<td>1-menaphthyl sulfate</td>
</tr>
<tr>
<td>Alkyl halides</td>
<td>2-chlorhydrin</td>
</tr>
<tr>
<td></td>
<td>1,2-epoxypropane</td>
</tr>
<tr>
<td>Alkene halides</td>
<td>Vinyl chloride</td>
</tr>
<tr>
<td>Organophosphorus compounds</td>
<td>Methylparathionine</td>
</tr>
<tr>
<td>Arene oxides</td>
<td>metabolites of naphthalene, benzo[a]pyrene</td>
</tr>
<tr>
<td>Steroids</td>
<td>$\Delta^5$-androstene-3,17-dione</td>
</tr>
</tbody>
</table>
“Chemoprevention involves administering specific amounts of a particular natural or synthetic chemical in an attempt to identify agents that will prevent, halt, or reverse the process of carcinogenesis.”
Cells contain various detoxification enzymes that neutralize cancer-causing chemicals before they can damage DNA.

Cancer occurs when these defenses are overwhelmed.

By **INDUCING** the detoxification enzymes, more effective protection can be achieved.
Enzyme Induction and chemoprevention

The discovery by Wattenberg in 1973 that food additive BHA protected rodents against carcinogens indeed provided the important stimulus to the development of the field of chemoprevention.

The follow up studies of the same agents by Talaly’s group in 1978 led to the startling discovery that administration of these antioxidants produced a marked change in the elevation of GST and other phase II enzymes.

A number of synthetic as well as natural chemical compounds are now known that causes induction of GSTs in many animal models.
The best sources of dietary antioxidants/Phase II enzyme inducers

Fruits

Vegetables
Biochemical and epidemiological studies indicate that intake of diet rich in fruits and vegetables is directly related to lower incidences of cancer.
ROLE OF GSH/GST-DEPENDENT DETOXIFICATION SYSTEM IN CHEMOPREVENTION

Working Hypothesis:
Effective inducers of Phase-II enzymes (GST and QR) should be good agents for chemoprevention
ENZYME INDUCTION AND CHEMOPREVENTION

Important Phase II Defense Enzymes

1. **Glutathione S-Transferase**: Inactivation of electrophiles by conjugation to GSH
2. **UDP-Glucorosyltransferase**: Inactivation of electrophiles by conjugation to glucoronic acid.
3. **NAD(P)H:Quinone oxidoreductase**: Catalyzes the obligatory two electron reduction of quinones and thus shields the cells against the electrophilicity of quinones and oxidative stress.
Induction of GST in various experimental models by synthetic antioxidants
Effect of BHT on the levels of GST in male and female mice

Sharma et al. 1993 Comp. Biochem. & Physiol 115C,31
Effect of BHT on GSH levels of rat lens cultured \textit{in vitro}

Ahmad et al. 1992 Exp. Eye Res. \textbf{54}, 41
Effect of BHT on Glutathione S-transferase levels of rat lens cultured *in vitro*

Ahmad *et al.* 1992 *Exp. Eye Res.* 54, 41
Myristicin
Inducer of GST

\[
\begin{align*}
\text{H}_2\text{C} \quad & \quad \text{OCH}_3 \\
\text{O} \quad & \quad \text{O} \\
\text{OCH}_3 \quad & \quad \text{O}
\end{align*}
\]
Biological source of Myristicin

carrots
dill
broccoli
coffee

nutmeg
Induction of GST in mice

- Appropriate amounts of myristicin were administered once every other day for a total of three doses.
- The animals were sacrificed 48 hours after the last dose.
Effect of Myristicin on the GST levels in mouse liver

1, 2, 3 & 4 = dose

A, B, C = substrates

Effect of Myristicin on Rat Small Intestine GST
Western Blots of Affinity Purified GST

Stomach

GST pi

1  C  E

1  Positive control
C  Control
E  Experimental

Small Intestine

GST pi

1  C  E

1  Positive control
C  Control
E  Experimental
Studies to investigate the effect of induction of GST by myristicin on various strains, age and gender in mice.
(unpublished data)
Increase in specific GST activity in mouse liver
Increase in specific GST activity in mouse livers

Fold Increase

CBA/J  BALB/c  C57BL/6  A/J
Increase in specific GST activity in mouse livers
Studies demonstrate that the Myristicin-induced GST is capable of metabolizing at least two known carcinogens.
Effect of Myristicin-induced GST against 4-nitroquinoline 1-oxide.
Effect of Myristicin-induced GST against Benzo(a)pyrene-4,5-oxide

GST Activity against BP-4,5-Oxide

<table>
<thead>
<tr>
<th>Units/mg of protein/minute</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.4</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Meristicine reduces the incidence of cancer in Benzo(a)Pyrene-induced carcinogenesis in mouse tissues.
Citrus and commercial flavonoids tend to selectively induce GST and QR in mice tissues.

Limonoids also selectively yet effectively induces the phase II enzymes in mouse organs.

Structures of the Phase-II enzyme inducers (phytochemicals) may be important in achieving optimal induction.
Specific induction of GST by Limonin -7-methoxime in

<table>
<thead>
<tr>
<th></th>
<th>Intestine</th>
<th>Liver</th>
<th>Lung</th>
<th>Stomach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione S-Transferase Activity (units/mg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limonin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limonin-7-methoxime</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Defuran limonin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limonin glucoside</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deacetyl nomilinic acid glucoside</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* * * *
Increase in specific GST activity in mouse livers cancer cells by ginger extracts
Increase in specific GST activity in mouse livers cancer cells by okra seed extracts

GST activity vs. 4-NQO in Hepa1c1c7

GST activity (units/mg)

- Media Control
- Positive Control
- Hot 8.26μg/μl
- Cold 5.50μg/μl
- Hot 0.826μg/μl
- Cold 0.55μg/μl
Increase in specific QR activity in mouse livers cancer cells by okra seed extracts

QR activity in Hepa 1c1c7

- Media Control
- Positive Control
- Hot 8.26μg/μl
- Cold 5.50μg/μl
- Hot 0.826μg/μl
- Cold 0.55μg/μl
Key Findings

- Myristicin induces GST levels in many mouse and rat tissues.

- Some carcinogens are efficiently metabolized by the induced GST.

- The induction of GST by myristicin in certain organs is affected by age, gender, or strain.
Key Findings

• In liver, there is appreciable increase in GST activity in most mice strains tested.

• In some cases the induction of phase II enzymes is tissue specific.
Future Direction

- To determine the signal transduction mechanism involved in the induction of phase-II enzymes.
- To identify more phytochemicals capable of inducing phase-II enzymes.
Trends in Consumption of Five or More Recommended Vegetable and Fruit Servings for Cancer Prevention, Adults 18 and Older, US, 1994-2009

![Bar chart showing prevalence of vegetable and fruit consumption from 1994 to 2009.](chart)

- **1994**: 24.2%
- **1996**: 24.4%
- **1998**: 24.1%
- **2000**: 24.4%
- **2003**: 23.6%
- **2005**: 24.3%
- **2007**: 24.7%
- **2009**: 23.8%

**Note:** Data from participating states and the District of Columbia were aggregated to represent the United States.

# The History of Cancer Treatment

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000 BC</td>
<td>Here, eat these <em>seeds</em></td>
</tr>
<tr>
<td>1000 BC</td>
<td>Seeds are heathen. Here, say this prayer.</td>
</tr>
<tr>
<td>1850 AD</td>
<td>Prayer is superstition! Here, have the surgeon cut it out.</td>
</tr>
<tr>
<td>1940 AD</td>
<td>There's more than surgery, add radiation.</td>
</tr>
<tr>
<td>1985 AD</td>
<td>There's more than surgery and radiation, add chemotherapy.</td>
</tr>
<tr>
<td>2013 AD</td>
<td>No better? Here, eat these <em>seeds</em>!</td>
</tr>
</tbody>
</table>
CREDIT

All past and current research students and collaborators

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National Institute of Health
Texas Higher Education Coordinating Board
United States Department of Agriculture
Howard Hughes Medical Institute
Thank You!!

For further information please contact

ahmadh@utpa.edu
(956) 665-3372