Role of Biomarkers and Surrogate Endpoints: Limitations of Serum Concentrations as a Predictor of Response

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Garlic Derived Sulfur Compounds

\[
\gamma\text{- glutamyl S-allylcysteine} \rightarrow S\text{-allylcysteine (SAC)}
\]

\[
\text{Alliin} \rightarrow \text{Alliinase} \rightarrow \text{Allicin}
\]

\[
\text{Decomposition}
\]

3-vinyl-[4H]-1,3-dithiin
2-vinyl-[4H]-1,3-dithiin
Diallyl sulfide
Diallyl disulfide
Diallyl trisulfide
Cellular Effects of Garlic Constituents

- DADS-induced apoptosis (HCT116)
  - ↑ intracellular calcium (Milner- 1996)
- DADS-induced G2/M arrest (HCT116)
  - ↓ Cdk1, ↑ p-Cdk1, ↓ Cdc25C, ↑ Cyclin B (Milner-1997)
- DADS-induced apoptosis (MDA-MB-231)
  - ↑ Bax, ↓ Bcl-xL, and caspase-3 activation (Nakagawa- 2001)
Cellular Effects of Garlic Constituents

- DADS-induced apoptosis (SH-SY5Y neuroblastoma)-
  \[ \text{↑ ROS, cytochrome c release, JNK activation, and activation of caspase-3 and -9 (Filomeni et al- 2003)} \]

- SAMC-induced G2/M arrest and apoptosis induction (SW480, HT-29)-
  \[ \text{microtubule depolymerization, JNK and caspase activation (Xiao et al- 2003)} \]
Allium vegetables and risk of prostate cancer: A population-based study.

Hsing AW, Chokkalingam AP, Gao YT, Madigan MP, Deng J, Gridley G, Fraumeni JF Jr. Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD

Epidemiologic and laboratory studies suggest that allium vegetables and garlic constituents have antitumor effects. In a population-based, case-control study conducted in Shanghai, China, we investigated the association between intake of allium vegetables, including garlic, scallions, onions, chives, and leeks, and the risk of prostate cancer. We administered in-person interviews and collected information on 122 food items from 238 case subjects with incident, histologically confirmed prostate cancer and from 471 male population control subjects. Men in the highest of three intake categories of total allium vegetables (>10.0 g/day) had a statistically significantly lower risk (odds ratio [OR] = 0.51, 95% confidence interval [CI] = 0.34 to 0.76; P(trend)<.001) of prostate cancer than those in the lowest category (<2.2 g/day). Similar comparisons between categories showed reductions in risk for men in the highest intake categories for garlic (OR = 0.47, 95% CI = 0.31 to 0.71; P(trend)<.001) and scallions (OR = 0.30, 95% CI = 0.18 to 0.51; P(trend)<.001). The reduced risk of prostate cancer associated with allium vegetables was independent of body size, intake of other foods, and total calorie intake and was more pronounced for men with localized than with advanced prostate cancer.
Structures of Organosulfides

\[ \text{CH}_2=\text{CH-CH}_2\text{-S-CH}_2\text{-CH=CH}_2 \quad \text{Diallyl sulfide (DAS)} \]

\[ \text{CH}_2=\text{CH-CH}_2\text{-S-S-CH}_2\text{-CH=CH}_2 \quad \text{Diallyl disulfide (DADS)} \]

\[ \text{CH}_2=\text{CH-CH}_2\text{-S-S-S-CH}_2\text{-CH=CH}_2 \quad \text{Diallyl trisulfide (DATS)} \]

\[ \text{CH}_3\text{-CH}_2\text{-CH}_2\text{-S-CH}_2\text{-CH}_2\text{-CH}_3 \quad \text{Dipropyl sulfide (DPS)} \]

\[ \text{CH}_3\text{-CH}_2\text{-CH}_2\text{-S-S-CH}_2\text{-CH}_2\text{-CH}_3 \quad \text{Dipropyl disulfide (DPDS)} \]
DATS Inhibits PC-3 Cell Proliferation

Xiao et al. Oncogene, 2004
DATS Treated PC-3 Cells Are Arrested in G2/M Phase of the Cell Cycle

(24 h exposure)

Xiao et al., Oncogene, In Press, 2005
DATS Treated PC-3 Cells Are Arrested in G2/M Phase of the Cell Cycle

Xiao et al., Oncogene, In Press, 2005
DATS-Induced G2/M Phase Arrest in Synchronized PC-3 Cells

Control

Nocodazole 0.1 µg/ml 24h

+ DATS

- DATS

1 hour

2 hours

4 hours

2n 4n

2n 4n

2n 4n
Normal Prostate Epithelial Cells Are Resistant to Cell Cycle Arrest by DATS

Xiao et al., Oncogene, In Press, 2005
Regulation of G₂/M Transition

ATM/ATR → Chk2/Chk1

Cdc25C

Cdk1

Cyclin B

Inactive

Cytoplasm

Nucleus

Wee-1

Cdk1

Cyclin B

Active

P

14-3-3

Cdc25B

Cdk1

Cyclin B

Active

Cdc25C

P

Myt-1

Cdk1

Cyclin B

Inactive

P

P

P

P

Mitosis

Cytoplasm

Active

P

P
DATS Inhibits Cdk1 activity

DATS (40 µM) - + kDa

Cyclin B1
Actin
1 2.8 Fold

Cdc25C
Actin
1 0.5 Fold

P-cdk1 (Tyr 15)
Actin
1 1.5 Fold

P-Cdc25C (Ser 216)
Actin
1 3.5 Fold

Cdk1 kinase activity (% of control)

Control  DATS, 8h
Nuclear Accumulation of Cyclin B1 in DATS Treated PC-3 Cells

Control (DMSO, 8h)  DATS (40 µM, 8 h)
DATS Arrests Cells in Mitosis

Control vs DATS (40 µM, 8h)

% of mitotic cells

Control          DATS

4%                20%
DATS Activates Chk2

**A**

- DATS (40 μM)
- Chk2
- Actin
- P-Chk2 (Thr 68)

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>0</th>
<th>1</th>
<th>4</th>
<th>16</th>
<th>24</th>
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<tbody>
<tr>
<td>Chk2</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Actin</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>P-Chk2</td>
<td></td>
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**B**

- 8 h exposure
- kDa
- DATS (µM)

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<tr>
<th>DATS (µM)</th>
<th>0</th>
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<tbody>
<tr>
<td>Chk2</td>
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<td>Actin</td>
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<tr>
<td>P-Chk2</td>
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Chk2 Is Dispensable for DATS-Induced G2/M Arrest

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>%G₀/G₁</th>
<th>%S</th>
<th>%G₂-M</th>
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<tbody>
<tr>
<td>Mock</td>
<td>DMSO</td>
<td>50 ± 5</td>
<td>20 ± 4</td>
<td>25 ± 2</td>
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<tr>
<td></td>
<td>DATS</td>
<td>12 ± 1*</td>
<td>18 ± 3</td>
<td>58 ± 5*</td>
</tr>
<tr>
<td>Control</td>
<td>DMSO</td>
<td>52 ± 4</td>
<td>17 ± 2</td>
<td>25 ± 1</td>
</tr>
<tr>
<td></td>
<td>DATS</td>
<td>17 ± 3*</td>
<td>17 ± 3</td>
<td>53 ± 7*</td>
</tr>
<tr>
<td>Chk2</td>
<td>DMSO</td>
<td>53 ± 4</td>
<td>17 ± 3</td>
<td>25 ± 1</td>
</tr>
<tr>
<td></td>
<td>DATS</td>
<td>18 ± 1*</td>
<td>18</td>
<td>54 ± 1*</td>
</tr>
</tbody>
</table>

Data are mean ± SE (n= 3). Similar results were observed in 2 experiments.
*Significantly different compared with control by one-way ANOVA (p < 0.05).
Chk2 is dispensable for DATS-induced Cdc25C and Cdk1 phosphorylation and Mitotic Arrest
G2/M Arrest in HCT-15 Cells
Transfected with WT and Mutant Chk2

DATS (40 µM, 8 h)

Chk2-WT

Chk2-T68A

DATS (8 h)
DATS Increases Chk1 Phosphorylation

A

DATS (40 µM)  

<table>
<thead>
<tr>
<th></th>
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<th>4</th>
<th>16</th>
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<tbody>
<tr>
<td>Chk1</td>
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<td>44</td>
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<td>Actin</td>
<td>56</td>
<td>56</td>
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P-Chk1 (Ser 317)  

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<td>Actin</td>
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B

8 h exposure

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<th>1</th>
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<tr>
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P-Chk1 (Ser 317)  

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<td>44</td>
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<tr>
<td>Actin</td>
<td>56</td>
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</table>
Chk1 Protein Depletion Fails to Overcome DATS-induced G2/M arrest

<table>
<thead>
<tr>
<th>DATS (40 µM)</th>
<th>mock</th>
<th>control</th>
<th>Chk1 siRNA</th>
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<tbody>
<tr>
<td>Chk1</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Actin</td>
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</tr>
<tr>
<td>kDa</td>
<td>56</td>
<td>44</td>
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<tr>
<td>P-Cdc25C (Ser 216)</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Actin</td>
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<td></td>
</tr>
<tr>
<td>kDa</td>
<td>55</td>
<td>44</td>
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<tr>
<td>P-Cdc2 (Tyr 15)</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Actin</td>
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<td></td>
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</tr>
<tr>
<td>kDa</td>
<td>34</td>
<td>44</td>
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<tr>
<td>Cyclin B1</td>
<td>-</td>
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<tr>
<td>Actin</td>
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<tr>
<td>kDa</td>
<td>62</td>
<td>44</td>
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</table>

Mock

Control siRNA

Chk1 siRNA

PI Fluorescence

Cell Count
Chk1 Protein Knockdown Attenuates DATS-Induced Histone H3 Phosphorylation

Chk1 siRNA

Mock

Control

DATS (40 µM)

control siRNA

7 ± 1

25 ± 3

6 ± 1

24 ± 3

7 ± 1

13 ± 2
Chk1 Protein Knockdown Inhibits DATS-Induced Mitotic Arrest

*Significantly different compared with control by one-way ANOVA (P < 0.05)
Conclusions

- **DATS causes G2 and M phase cell cycle arrest due to:**
  - Even though Chk1 protein knockdown inhibits DATS induced phosphorylation of Cdc25C, G$_2$ arrest is not attenuated.
  - Chk1 depletion inhibits DATS-induced M phase arrest (securin, APC?)

- **DATS induces apoptosis by:**
  - Increasing JNK/ERK mediated phosphorylation of Bcl-2
  - Inhibiting PI3K/Akt pathway
Future Directions

- Mechanism of Chk1-dependent mitotic arrest (securin, Cdc20, APC?).
- Mechanism of DATS induced JNK activation (glutaredoxin/thioredoxin-ASK1?)
- Activity of DATS against prostate tumorigenesis in TRAMP model.
- Toxicological evaluation
- Pharmacokinetics
- Clinical Trial
Thanks to

- CA55589-07, CA76348-06, CA101753-01
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- Dr. Jedrzej Antosiewicz
- Kamayani Singh