Cruciferous Vegetables-Based Isothiocyanate Compounds as Novel Epigenetic Modulators in Human Malignant Melanoma

Mihalis Panagiotidis, PhD
Senior Scientist & Department Head

Department of Electron Microscopy & Molecular Pathology, Cyprus Institute of Neurology & Genetics, Nicosia, Cyprus
General Features of ITCs

- Cruciferous vegetables contain vitamins, minerals, other nutrients, and chemicals known as glucosinolates
- Glucosinolates break down into several biologically active compounds (e.g. ITCs) which are being studied for possible anticancer effects
- Some of them have shown anti-cancer effects in cells and animals but studies in humans have been inconclusive

![Image showing glucosinolate breakdown]

Myrosinase released by chopping or chewing cruciferous vegetables breaks down glucosinolates to isothiocyanates, releasing glucose and sulfate in the process. “R” designates side chains of other elements in the molecule. 

C=carbon, S=sulfur, N=nitrogen
**Major Dietary ITCs Of Interest**

Mitsiogianni et al. Pharmacol Ther, 190: 187-201, 2018

<table>
<thead>
<tr>
<th>ITC</th>
<th>GL-ITC Precursor</th>
<th>Food Source</th>
<th>Total Conc. (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFN</td>
<td>Glucoraphanin</td>
<td>Broccoli, Brussel sprouts, Cabbage</td>
<td>61 236 78</td>
</tr>
<tr>
<td>IBN</td>
<td>Glucoiberin</td>
<td>Cabbage, Broccoli</td>
<td>229 70</td>
</tr>
<tr>
<td>BITC</td>
<td>Glucotropaeolin</td>
<td>Cabbage, Garden cress</td>
<td>78 392</td>
</tr>
<tr>
<td>PEITC</td>
<td>Gluconasturtiiin</td>
<td>Watercress</td>
<td>94</td>
</tr>
<tr>
<td>AITC</td>
<td>Sinigrin</td>
<td>Broccoli, Brussel sprouts, Cabbage, Mustard greens</td>
<td>61 236 78 282</td>
</tr>
</tbody>
</table>
Modulation Of Various Cellular Pathways By ITCs

- Inhibition of Phase I and induction of Phase II enzymes

- Induction of cell cycle arrest (Mantso et al., Anticancer Research 36: 6303-6309, 2016)

- Promotion of apoptosis (Mantso et al., Anticancer Research, 2019, in press)

- Modulation of epigenetic response
  - HDAC inhibition
  - DNMTs inhibition
  - MicroRNA modulation

- Anti-angiogenic and anti-metastatic properties

Mitsiogianni et al. Antioxidants, 8(4): 106, 2019
In Vitro Model of Human Malignant Melanoma

- HaCaT cells
- A375 cells
- A431 cells
- VMM1 cells
- HS294T cells
Toxicity Profile of AITC


<table>
<thead>
<tr>
<th></th>
<th>A375</th>
<th>HaCaT</th>
<th>A431</th>
<th>VMM1</th>
<th>Hs294T</th>
<th>B16-F10</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC50&lt;sub&gt;24h&lt;/sub&gt; (μM)</td>
<td>15.6 ± 4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>21.7 ± 4.1</td>
<td>ND</td>
</tr>
<tr>
<td>EC50&lt;sub&gt;48h&lt;/sub&gt; (μM)</td>
<td>12 ± 0.7</td>
<td>ND</td>
<td>43.4 ± 15.8</td>
<td>ND</td>
<td>21.3 ± 6</td>
<td>14.9 ± 3.7</td>
</tr>
</tbody>
</table>
## Toxicity Profile of SFN, IBN, BITC & PEITC

<table>
<thead>
<tr>
<th></th>
<th>A375</th>
<th>HaCaT</th>
<th>A431</th>
<th>VMM1</th>
<th>Hs294T</th>
<th>B16-F10</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC50$_{24h}$ (μM)</td>
<td>15.7 ± 0.15</td>
<td>21.6 ± 2.00</td>
<td>46.05 ± 12.04</td>
<td>35.3 ± 11.7</td>
<td>8.97 ± 0.8</td>
<td>20.88 ± 2.00</td>
</tr>
<tr>
<td>EC50$_{48h}$ (μM)</td>
<td>15.6 ± 2.4</td>
<td>23.3 ± 2.4</td>
<td>20.87 ± 0.96</td>
<td>24.11 ± 1.3</td>
<td>6.5 ± 0.12</td>
<td>15.37 ± 0.5</td>
</tr>
<tr>
<td>IBN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC50$_{24h}$ (μM)</td>
<td>8.3 ± 1.06</td>
<td>23.56 ± 4.4</td>
<td>48.51 ± 16.1</td>
<td>50.5 ± 9.5</td>
<td>11.3 ± 2.6</td>
<td>21.09 ± 0.9</td>
</tr>
<tr>
<td>EC50$_{48h}$ (μM)</td>
<td>8.6 ± 0.7</td>
<td>23.6 ± 1.9</td>
<td>15.26 ± 1.8</td>
<td>23.05 ± 1.2</td>
<td>8.96 ± 1.6</td>
<td>19.97 ± 5.4</td>
</tr>
</tbody>
</table>


![SFN](image1)

![IBN](image2)
Overview Of Cancer Epigenetics

Epigenetics (above genetics)
Stable and heritable changes in gene expression and chromatin organization but independent of the DNA coding sequence itself.
The Concept of Epigenetic Diet: How What We Eat Could Affect Tags on Our DNA
Dietary Inhibitors of DNA Methyltransferases

- Hypermethylation of CpG dinucleotides, by DNMTs, usually results in transcriptional gene silencing and gene inactivation.
- Several bioactive compounds act as dietary inhibitors of DNA methyltransferases and also alter gene expression via epigenetic mechanisms.

Hardy, TM & Tollefsbol, TO. Epigenomics, 3(4): 503-518, 2011
Bioactive compounds can alter HATs and HDACs by causing conformational changes in chromatin structure thereby altering gene expression.

HATs induce a relaxed chromatin state indicative of gene expression whereas chromatin in its closed state is indicative of gene silencing and repression.

Hardy, TM & Tollefsbol, TO. Epigenomics, 3(4): 503-518, 2011
<table>
<thead>
<tr>
<th>DIETARY COMPOUNDS</th>
<th>FOOD SOURCES</th>
<th>EPIGENETIC FUNCTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC, ECG, EGC and EGCG</td>
<td>Green tea</td>
<td>DNMT and HAT inhibitor, modulates miRNA</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>Grapes, peanuts, mulberries, cranberries, blueberries</td>
<td>DNMT and HDAC inhibitor</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Tumeric, curry</td>
<td>DNMT inhibitor and miRNA modulator</td>
</tr>
<tr>
<td>Genistein</td>
<td>Soybeans, fava beans</td>
<td>DNMT and HDAC inhibitor, enhances HATs, modulates miRNA</td>
</tr>
<tr>
<td>Isothiocyanates, sulforaphane</td>
<td>Broccoli, cabbage, kale, watercress</td>
<td>DNMT and HDAC inhibitor</td>
</tr>
<tr>
<td>Selenium</td>
<td>Brazilian nuts, chicken, game meat, beef</td>
<td>DNMT and HDAC inhibitor</td>
</tr>
<tr>
<td>Allyl mercaptan, organosulfur compounds</td>
<td>Garlic</td>
<td>HDAC inhibitor</td>
</tr>
<tr>
<td>Folate</td>
<td>Beans, grains, fortified breakfast cereals, pastas, green vegetables</td>
<td>Deficiencies alter DNA methylation patterns</td>
</tr>
<tr>
<td>Alcohol</td>
<td>Alcoholic beverages</td>
<td>High consumption increases promoter hypermethylation</td>
</tr>
</tbody>
</table>

*Hardy, TM & Tollefsbol, TO. Epigenomics, 3(4): 503-518, 2011*
Histone Modifications Capable of Altering Gene Expression

- Histone N-terminal tail has a crucial role in modulating nucleosome structure and function.
- Various modifications on different residues of histone tail are shown.
- S, T, K and R represent Serine, Threonine, Lysine & Arginine respectively.

AITC Induces The Epigenetic Response

SFN & IBN Induce The Epigenetic Response

Inhibition Of DNMT1A Potentiates the Effect of AITC

If a tumor-suppressor gene is not being transcribed in a malignant cell, treating with a DNA-methyltransferase inhibitor can open the chromatin and change the level at which transcription is occurring, thereby killing the malignant cell. This is the hypothesis for how Azacytidine and Decitabine are working in MDS.

Pre-treatment with Decitabine, for 24 hrs, followed by co-treatment with AITC and Decitabine for 48 hrs.

Inhibition Of HDAC Potentiates the Effect of AITC

Pre-treatment with Panobinostat, for 24 hrs, followed by co-treatment with AITC and Panobinostat for 48 hrs

Inhibition Of HAT Potentiates the Effect of AITC

Pre-treatment with Anacardic Acid, for 24 hrs, followed by co-treatment with AITC and Anacardic Acid for 48 hrs

www.researchgate.net

Epigenetic Therapy & HDACi

Modelling of ITCs as HDAC inhibitors by computer-aided drug design

Order of HDAC inhibition by SFN in vitro:
SFN-Cys > SFN-NAC > SFN-GSH >> SFN


Epigenetic Therapy & HDACi

**Invest New Drugs.** 2012 Dec;30(6):2303-2317.
Phase 1 clinical trial of the novel proteasome inhibitor Marizomib with the histone deacetylase inhibitor Vorinostat in patients with melanoma, pancreatic and lung cancer based on in vitro assessments of the combination.

Phase I trial of Vorinostat and Doxorubicin in solid tumours: histone deacetylase 2 expression as a predictive marker.

Multi-center phase II trial of the histone deacetylase inhibitor Entinostat in pre-treated metastatic melanoma.

**Clin Cancer Res.** 2009 Apr 1;15(7):2479-2487.
Potentiation of a topoisomerase I inhibitor, Karenitecin, by the histone deacetylase inhibitor Valproic acid in melanoma: translational and phase I/II clinical trial.

**Br J Cancer.** 2009 Jan 13;100(1):28-36.
A phase I-II study of the histone deacetylase inhibitor Valproic acid plus Dacarbazine and Interferon-α in patients with advanced melanoma.

**Mottamal et al. Molecules, 20(3): 3898-941, 2015**
**Acknowledgements**

**Current and Past Lab Members**
- Dr Theodora Mantso
- Dr Melinna Mitsiogianni
- Dr Natalia Ferreira
- Dr Ioannis Anestopoulos
- Ms Venetia Tragkola
- Ms Eleni Tzika
- Dr Sotiris Kyriakou

**Network of Collaborators**
- Drs Pappa, Botaitis, Chlichlia
- Dr Trafalis
- Drs Tetard, Kazhevnikov, Birkett, Veuger
- Prof Zoumpourlis
- Prof Rupasinghe
- Mr Amery
- Dr Bronowska
- Dr Franco
- Prof Ulukaya

**Funding Agencies**

European Union
Ministry of Education, Lifelong Learning and Religious Affairs
MANAGING AUTHORITY
Co-financed by Greece and the European Union

The Leverhulme Trust
The Watercress Company
Dalhousie University
ISU IETINDEI UNISTI
NSRF 2007-2013
The Framwork Programme for Research and Innovation