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

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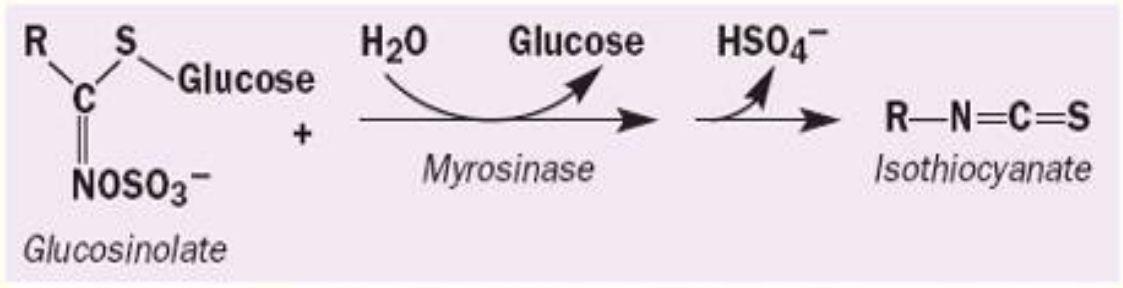
General Features of ITCs

- 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)



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

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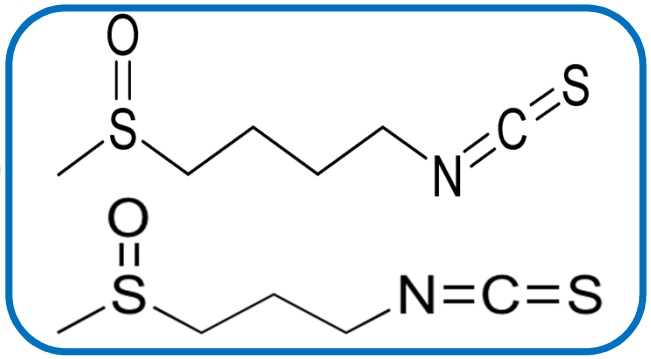
Major Dietary ITCs Of Interest

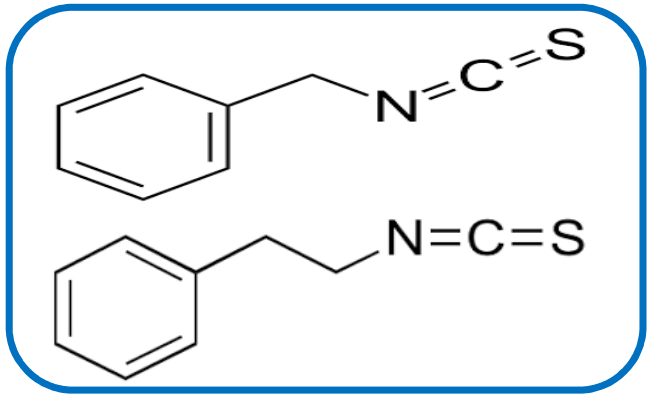
Sulforaphane (SFN)

Iberin (IBN)

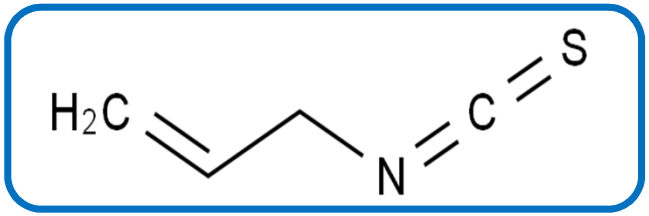


Benzyl-ITC (BITC)

Phenethyl-ITC (PEITC)



Allyl-ITC (AITC)



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

ITC	GL-ITC Precursor	Food Source	Total Conc. (mg/100g)
SFN	Glucoraphanin	Broccoli Brussel sprouts Cabbage	61 236 78
IBN	Glucoiberin	Cabbage Broccoli	229 70
BITC	Glucotropaeolin	Cabbage Garden cress	78 392
PEITC	Gluconasturtiin	Watercress	94
AITC	Sinigrin	Broccoli Brussel sprouts Cabbage Mustard greens	61 236 78 282

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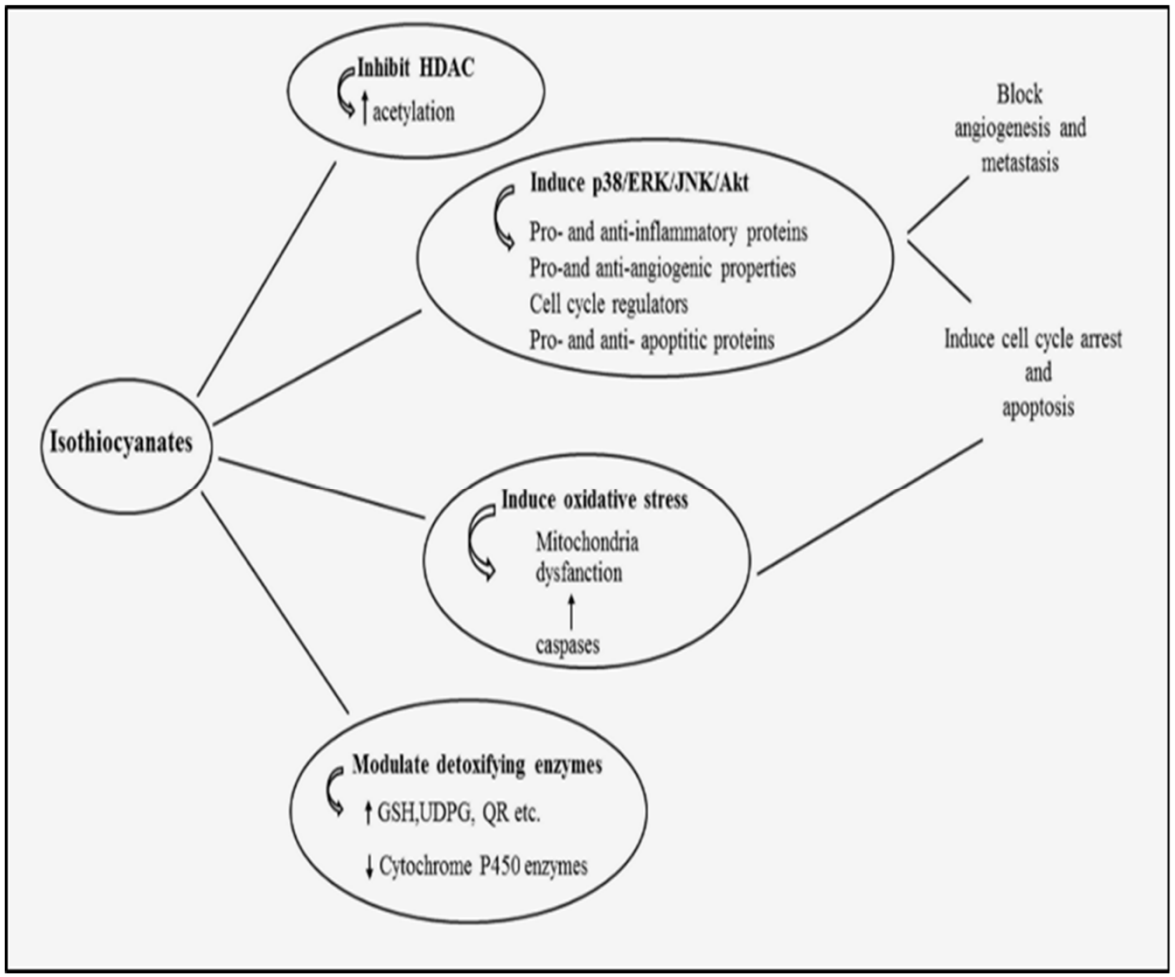
Modulation Of Various Cellular Pathways By ITCs



Mitsiogianni et al. Antioxidants, 8(4): 106, 2019

- **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**

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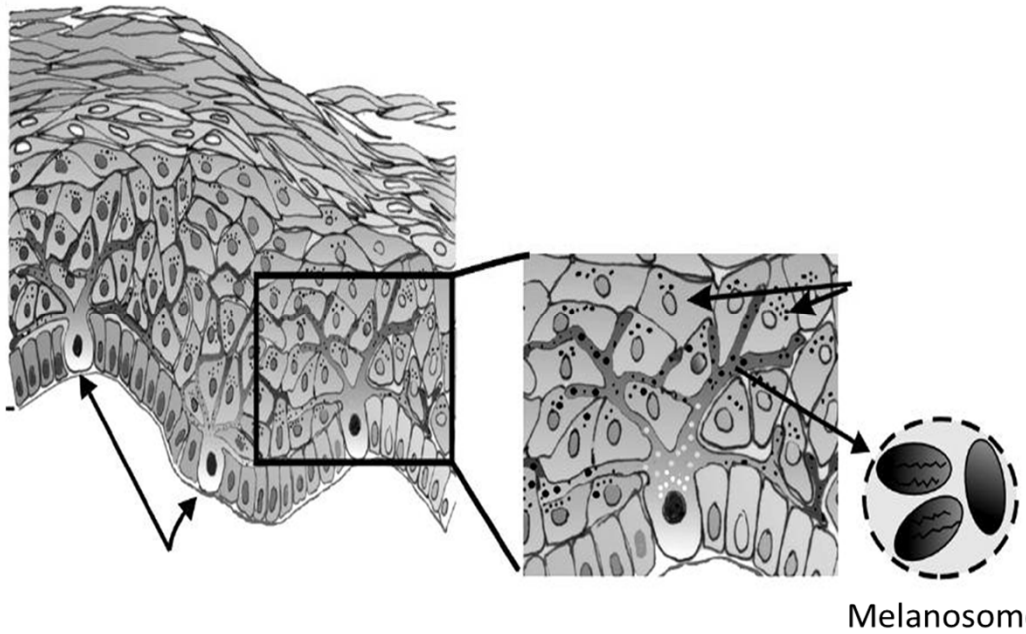
In Vitro Model of Human Malignant Melanoma

Stratum corneum

Stratum granulosum

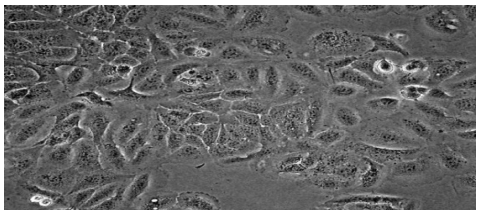
Stratum spinosum

Stratum germinativum (basal layer)

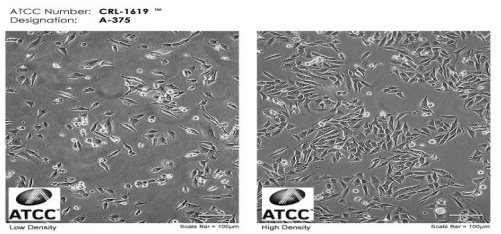


Melanosome

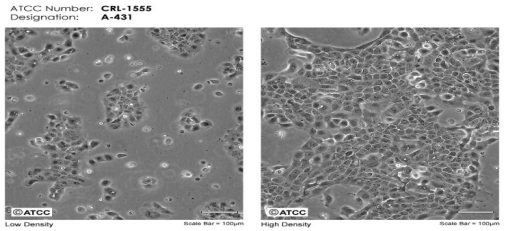
➢ **HaCaT cells**



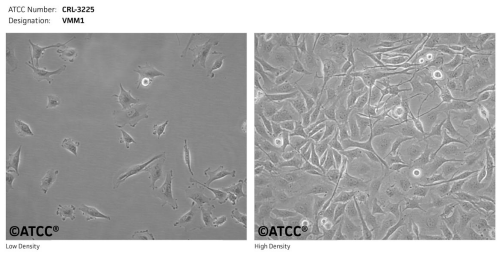
➢ **A375 cells**



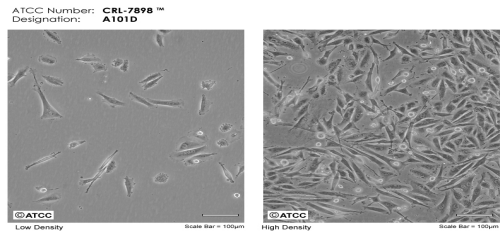
➢ **A431 cells**



➢ **VMM1 cells**



➢ **HS294T cells**



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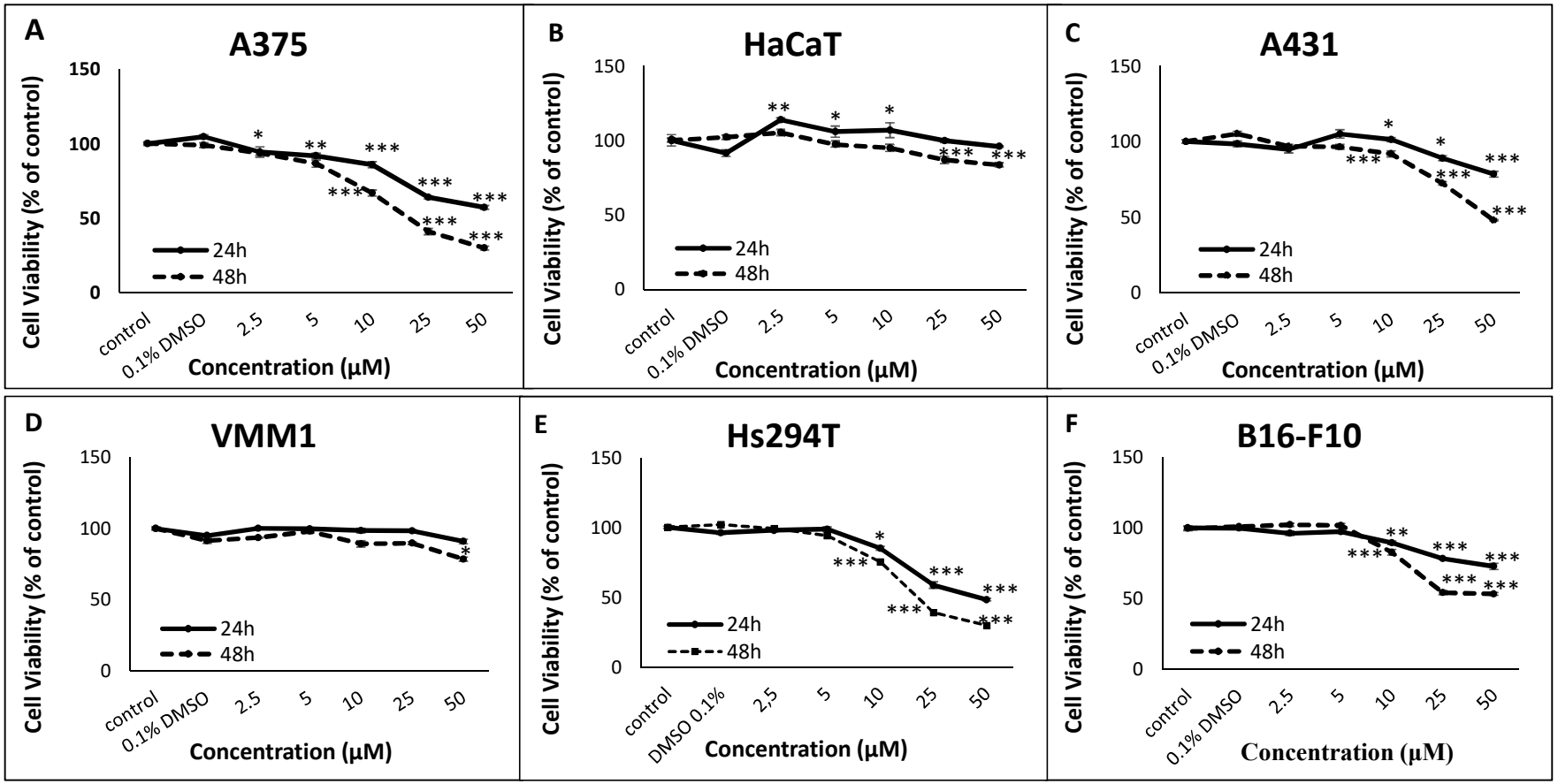
Toxicity Profile of AITC



	A375	HaCaT	A431	VMM1	Hs294T	B16-F10
$EC50_{24h}$ (μM)	15.6 ± 4	ND	ND	ND	21.7 ± 4.1	ND
$EC50_{48h}$ (μM)	12 ± 0.7	ND	43.4 ± 15.8	ND	21.3 ± 6	14.9 ± 3.7

Mitsiogianni et al. Eur J Nutr, 59(2): 557-669, 2020

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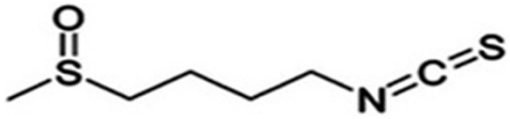
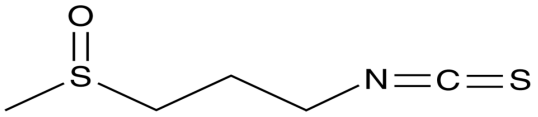
Toxicity Profile of SFN, IBN, BITC & PEITC

		A375	HaCaT	A431	VMM1	Hs294T	B16-F10
SFN	$EC50_{24h}$ (μM)	15.7 ± 0.15	21.6 ± 2.00	46.05 ± 12.04	35.3 ± 11.7	8.97 ± 0.8	20.88 ± 2.00
	$EC50_{48h}$ (μM)	15.6 ± 2.4	23.3 ± 2.4	20.87 ± 0.96	24.11 ± 1.3	6.5 ± 0.12	15.37 ± 0.5
IBN	$EC50_{24h}$ (μM)	8.3 ± 1.06	23.56 ± 4.4	48.51 ± 16.1	50.5 ± 9.5	11.3 ± 2.6	21.09 ± 0.9
	$EC50_{48h}$ (μM)	8.6 ± 0.7	23.6 ± 1.9	15.26 ± 1.8	23.05 ± 1.2	8.96 ± 1.6	19.97 ± 5.4



SFN



IBN

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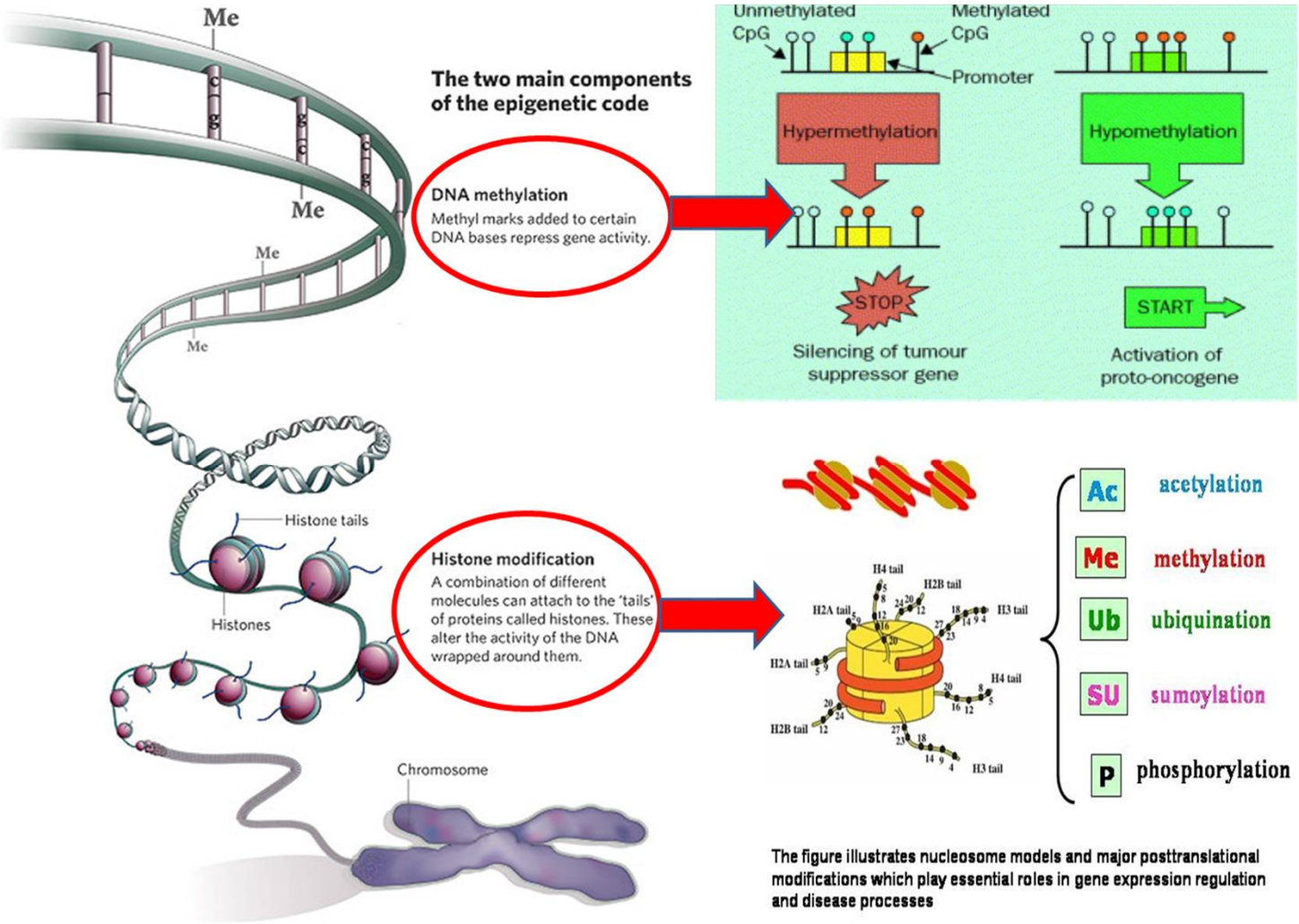
Overview Of Cancer Epigenetics

Epigenetics (above genetics)

Stable and heritable changes in gene expression and chromatin organization but independent of the DNA coding sequence itself



The figure illustrates nucleosome models and major posttranslational modifications which play essential roles in gene expression regulation and disease processes

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The Concept of Epigenetic Diet: How What We Eat Could Affect Tags on Our DNA

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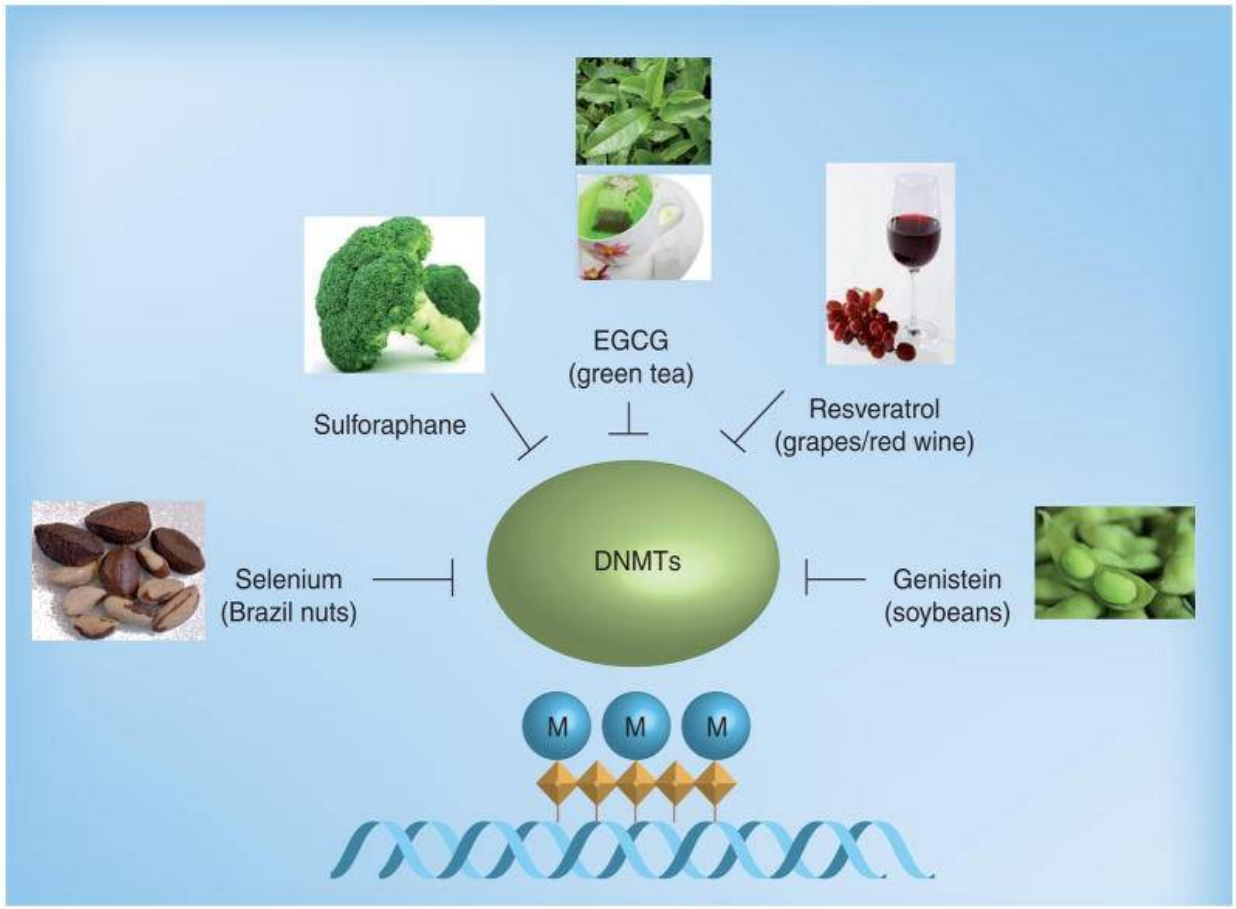
Dietary Inhibitors of DNA Methyltransferases



- **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

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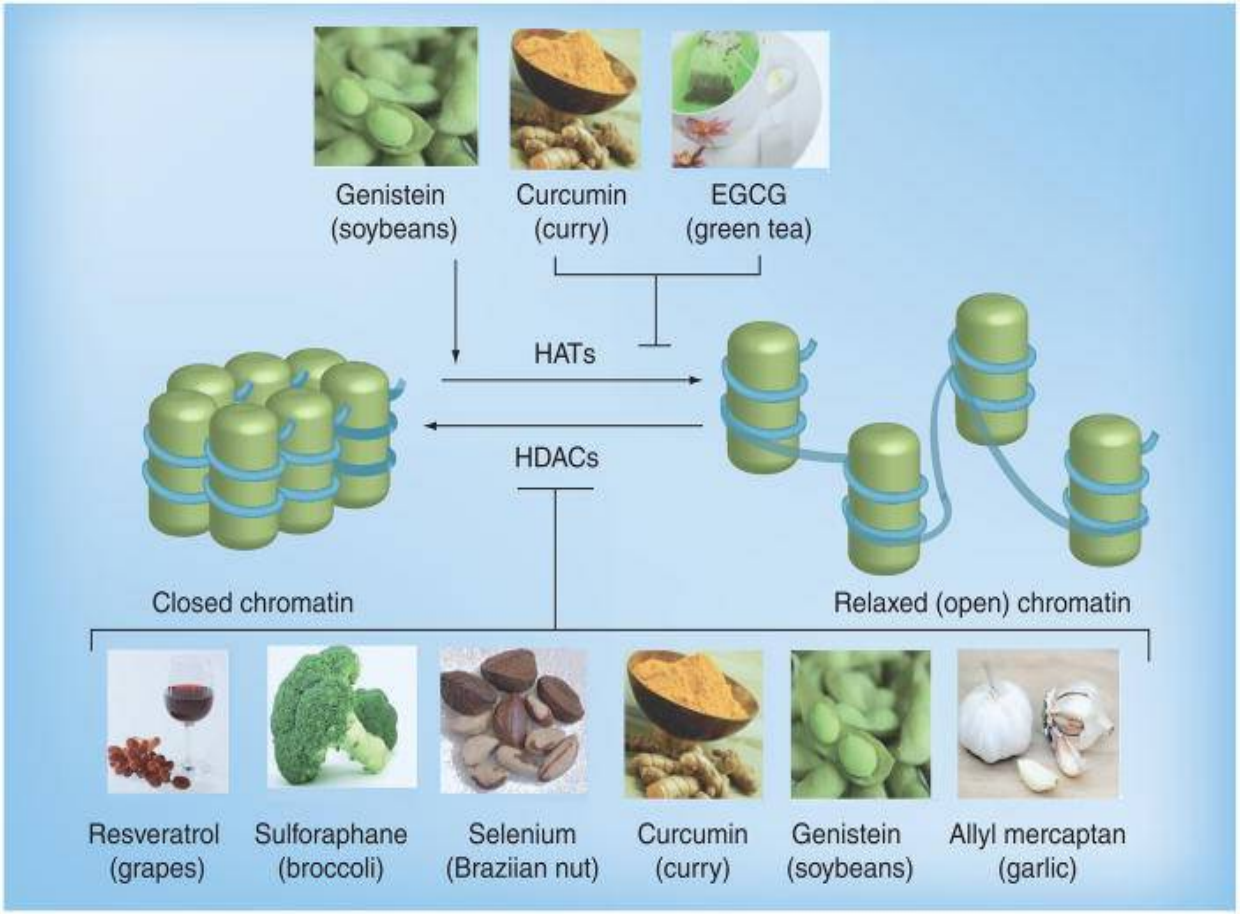
Dietary Modifiers of Histones



- **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

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DIETARY COMPOUNDS	FOOD SOURCES	EPIGENETIC FUNCTIONS
EC, ECG, EGC and EGCG	Green tea	DNMT and HAT inhibitor, modulates miRNA
Resveratrol	Grapes, peanuts, mulberries, cranberries, blueberries	DNMT and HDAC inhibitor
Curcumin	Tumeric, curry	DNMT inhibitor and miRNA modulator
Genistein	Soybeans, fava beans	DNMT and HDAC inhibitor, enhances HATs, modulates miRNA
Isothiocyanates, sulforaphane	Broccoli, cabbage, kale, watercress	DNMT and HDAC inhibitor
Selenium	Brazilian nuts, chicken, game meat, beef	DNMT and HDAC inhibitor
Allyl mercaptan, organosulfur compounds	Garlic	HDAC inhibitor
Folate	Beans, grains, fortified breakfast cereals, pastas, green vegetables	Deficiencies alter DNA methylation patterns
Alcohol	Alcoholic beverages	High consumption increases promoter hypermethylation

Hardy, TM & Tollefsbol, TO. Epigenomics, 3(4): 503-518, 2011

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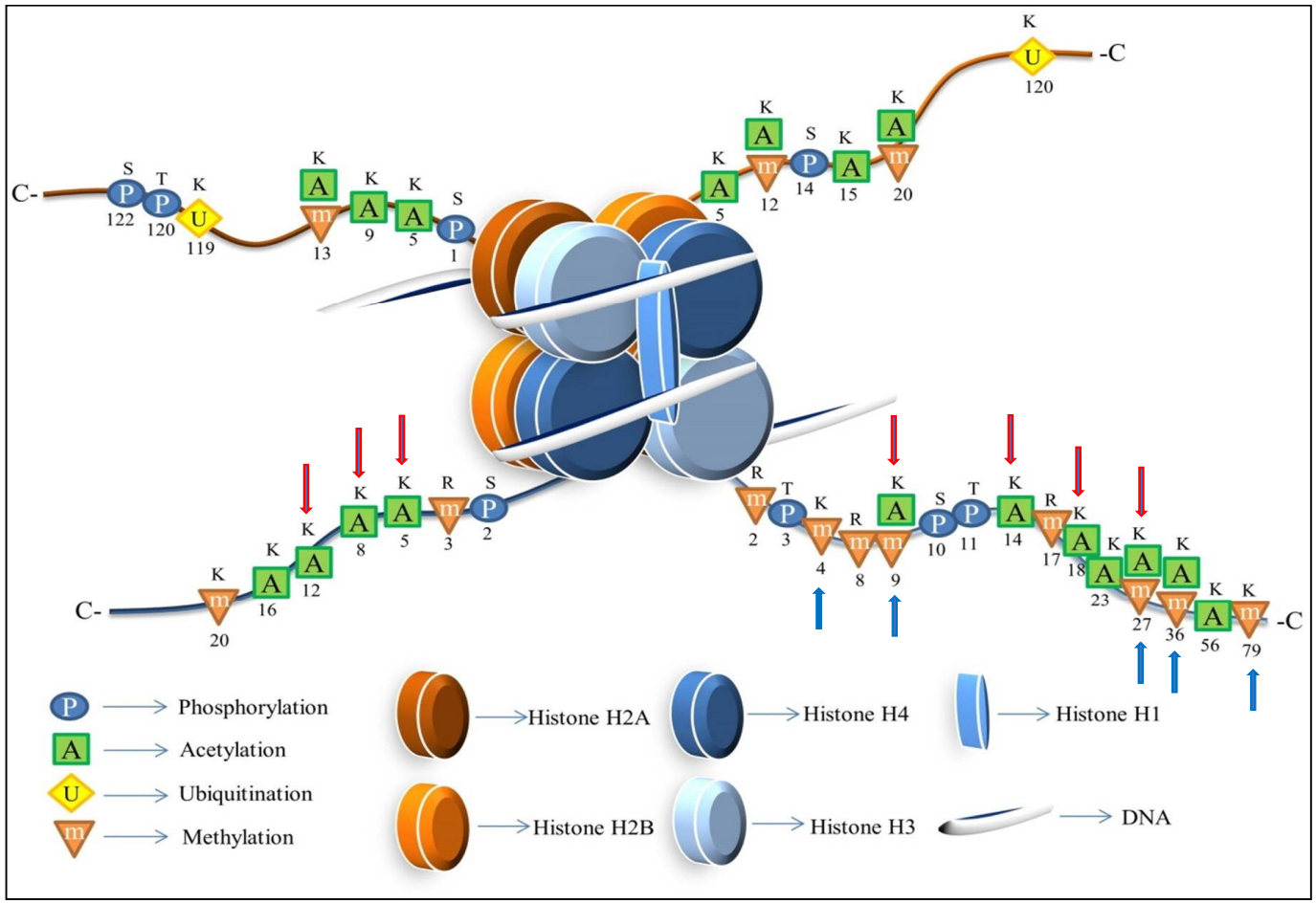
Histone Modifications Capable of Altering Gene Expression



- 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

Biswas, S & Rao, CM. *Pharmacol Ther*, 173: 118-134, 2017

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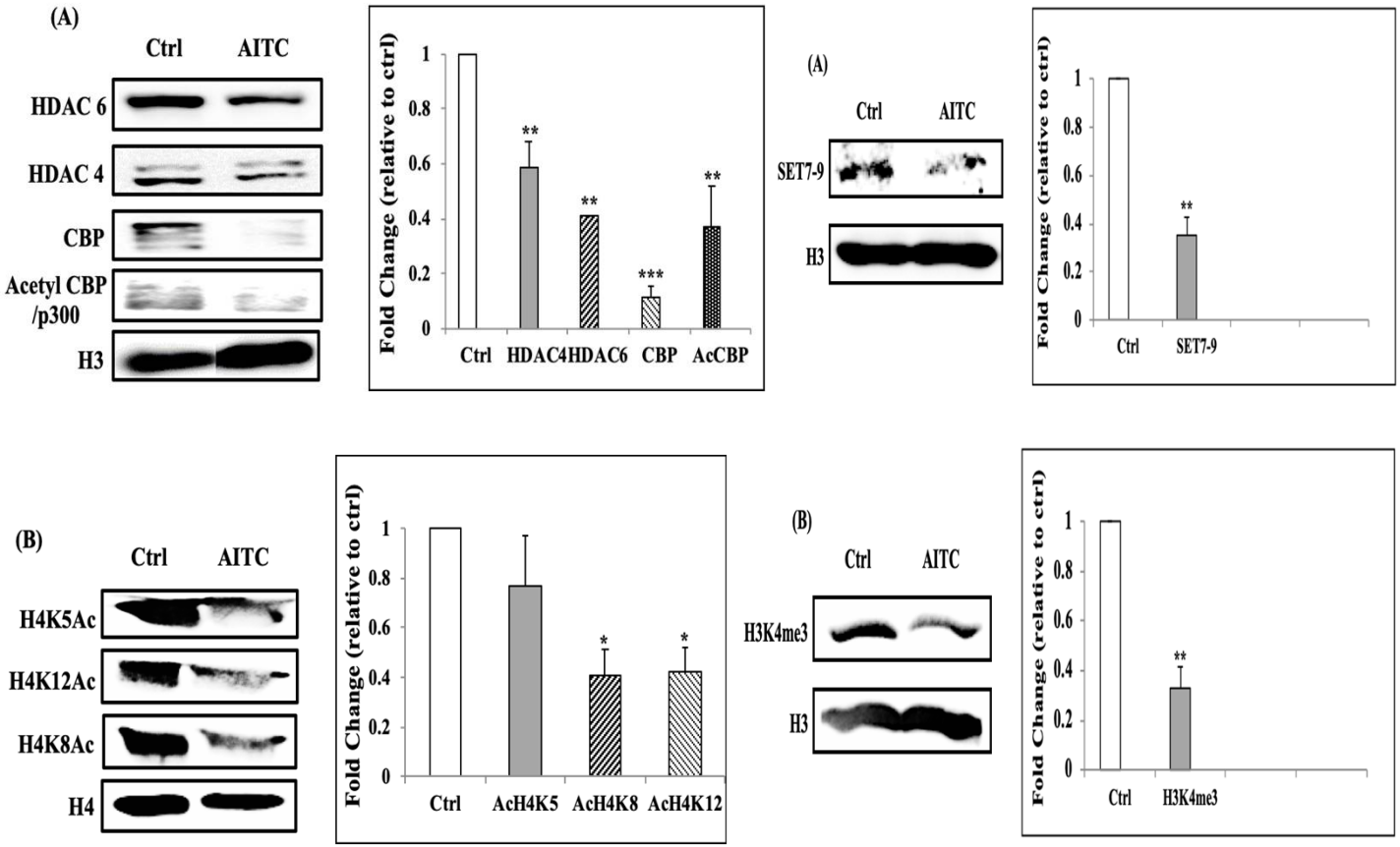
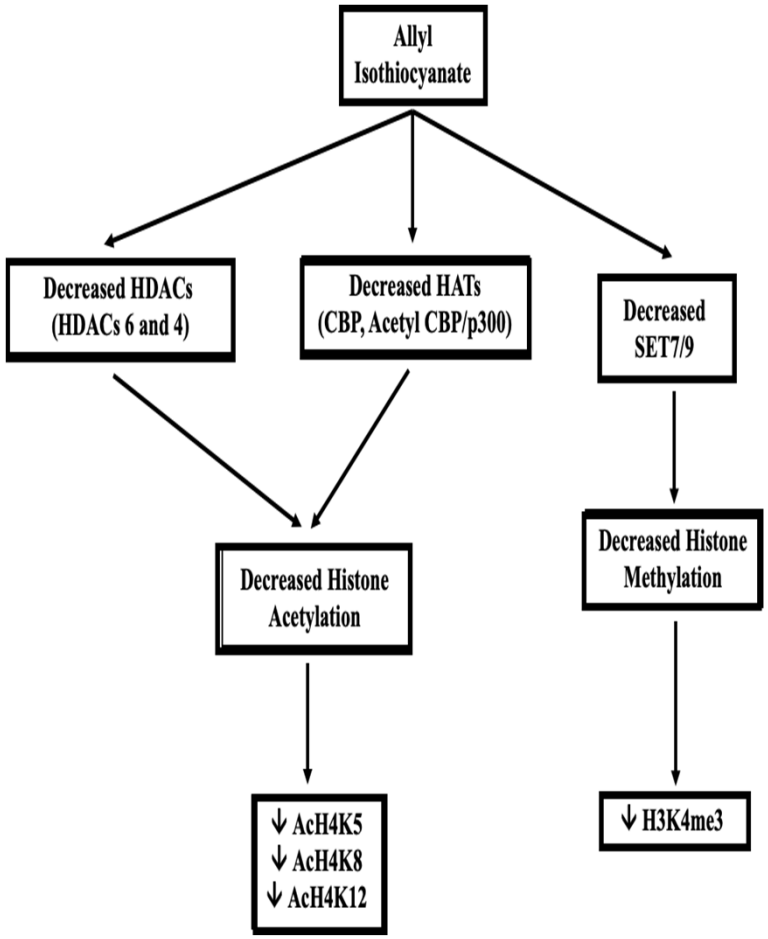
AITC Induces The Epigenetic Response





Mitsiogianni et al. Eur J Nutr, 59(2): 557-669, 2020

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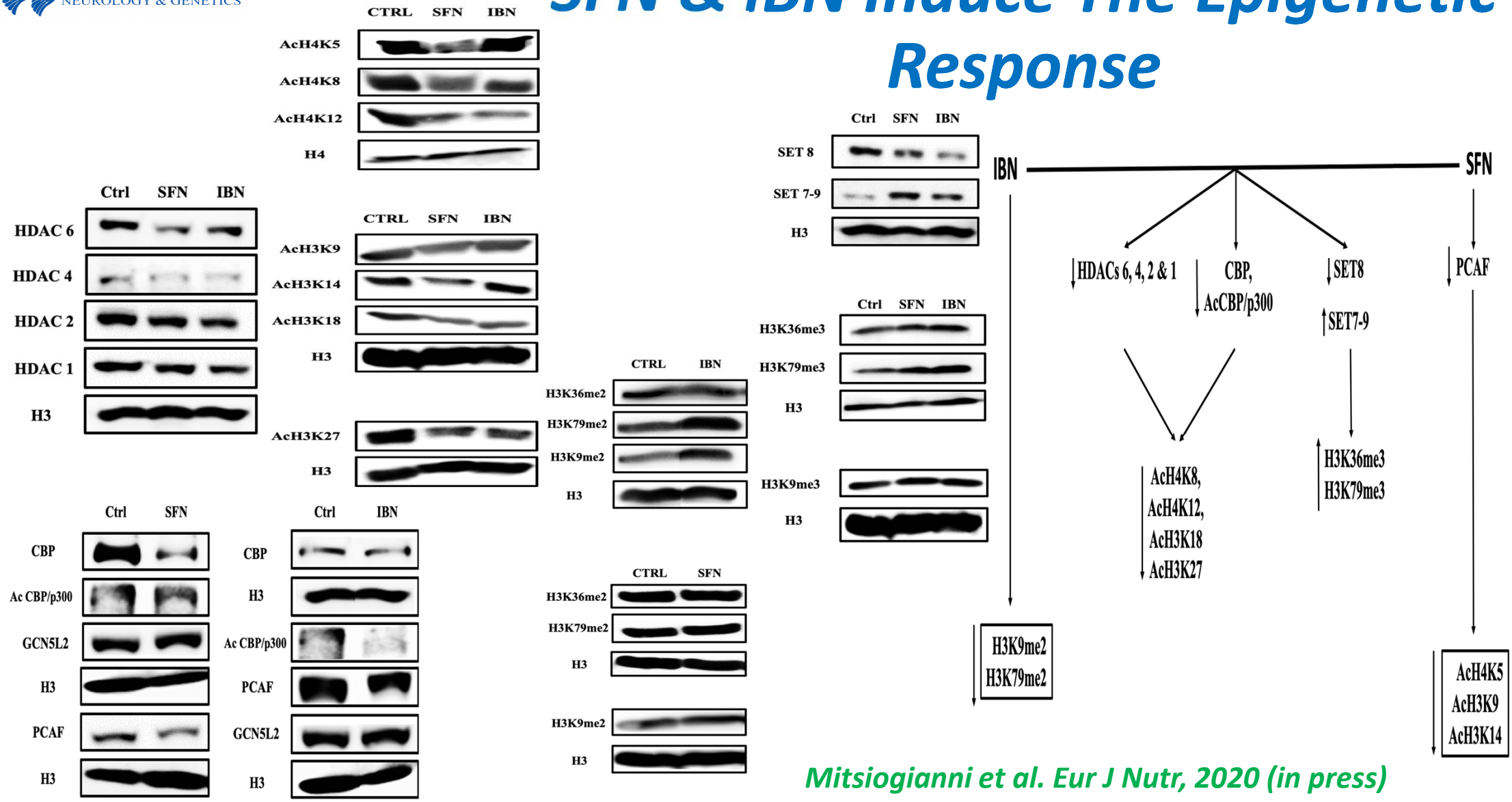
SFN & IBN Induce The Epigenetic Response



Mitsiogianni et al. Eur J Nutr, 2020 (in press)

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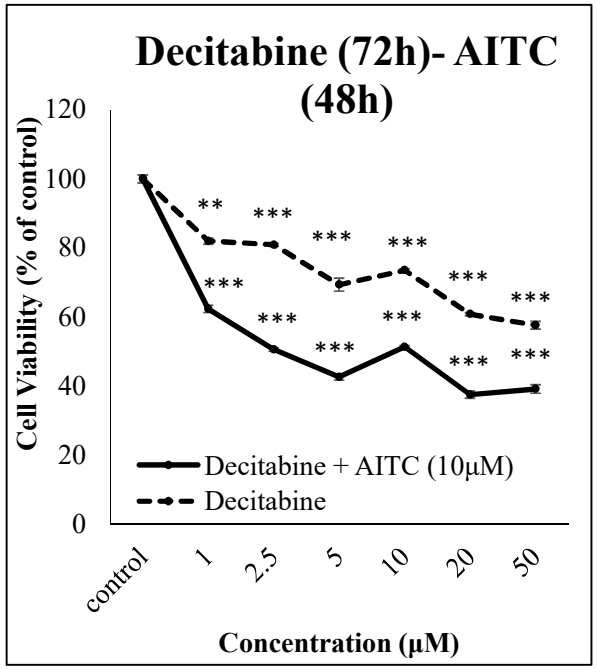
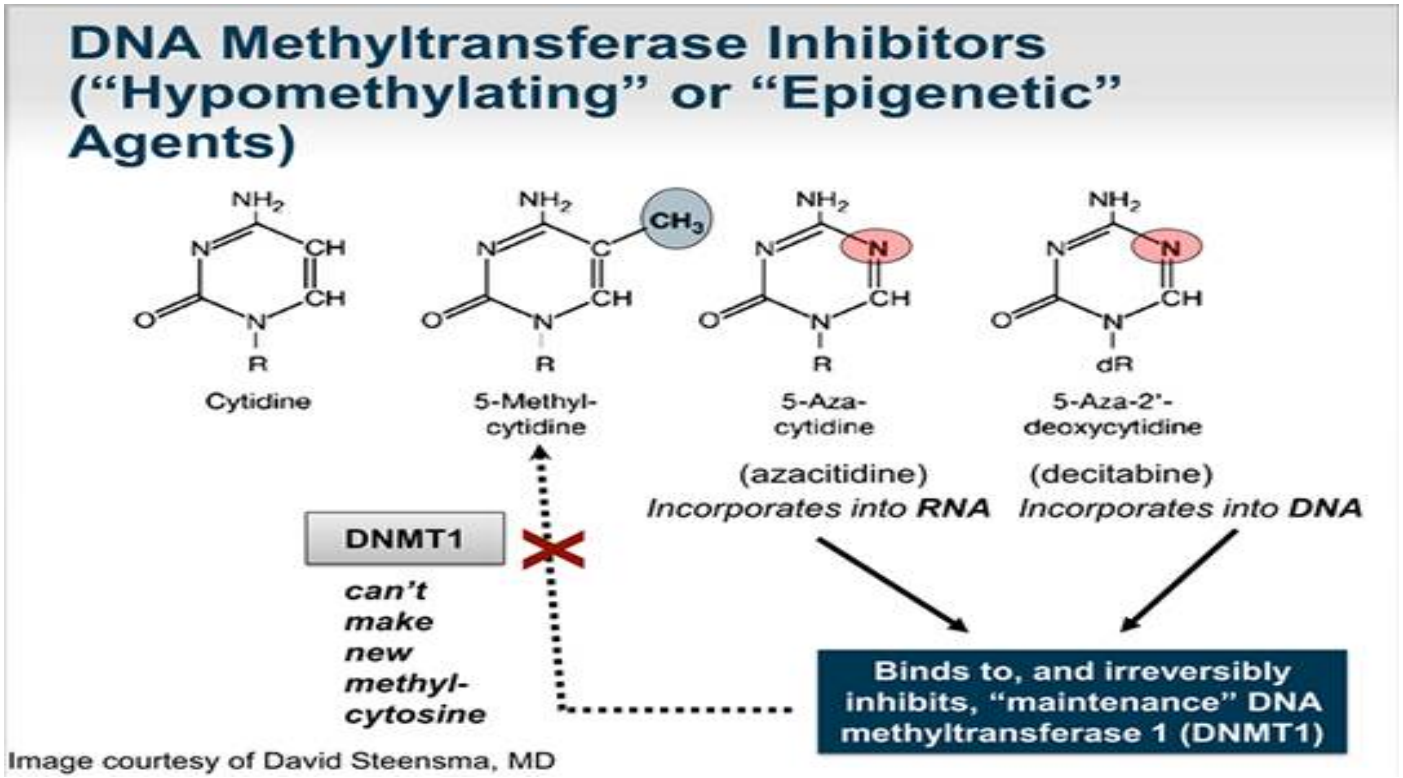
Inhibition Of DNMT1A Potentiates the Effect of AITC



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



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

Mitsiogianni et al. Eur J Nutr, 59(2): 557-669, 2020

www.medscape.org

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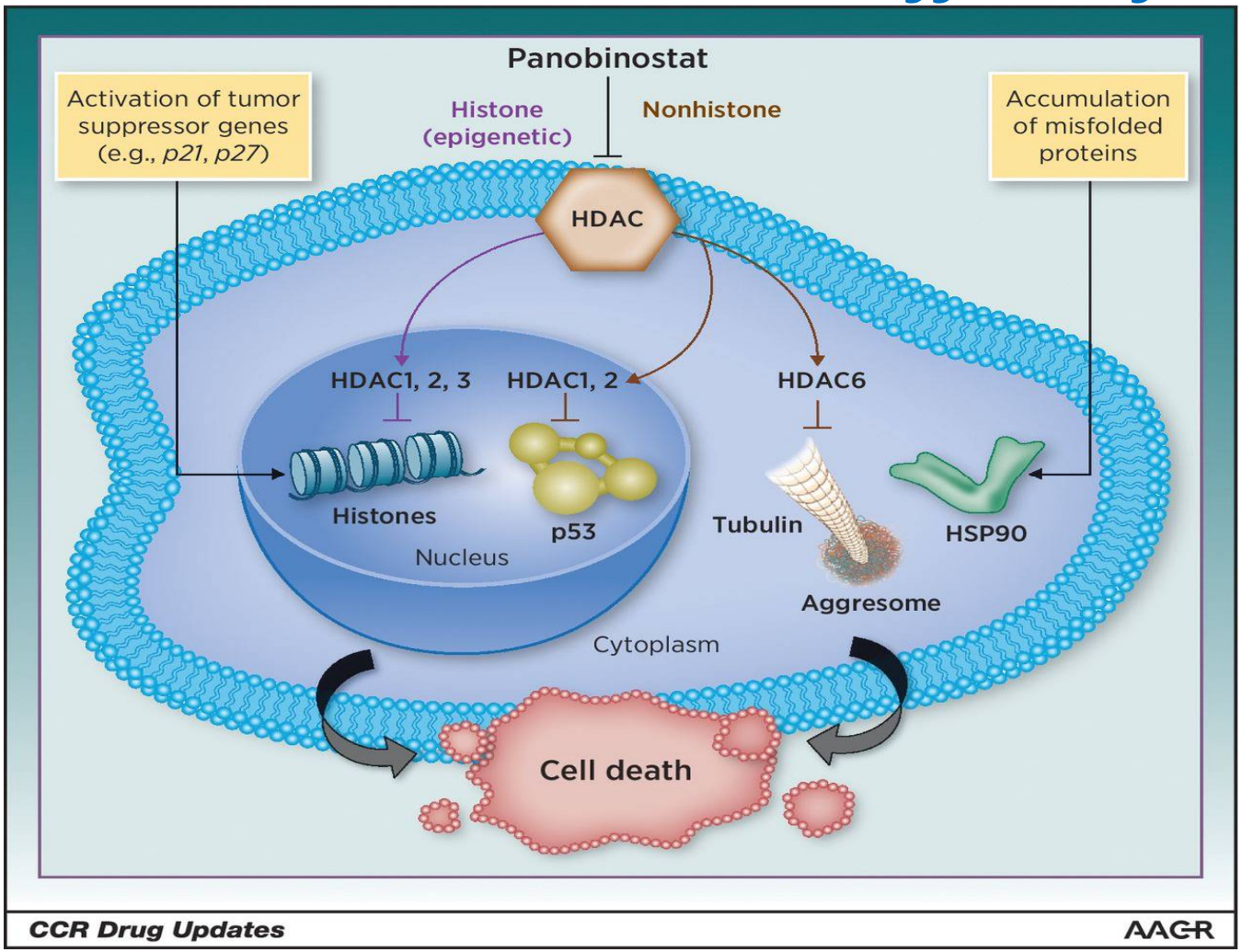
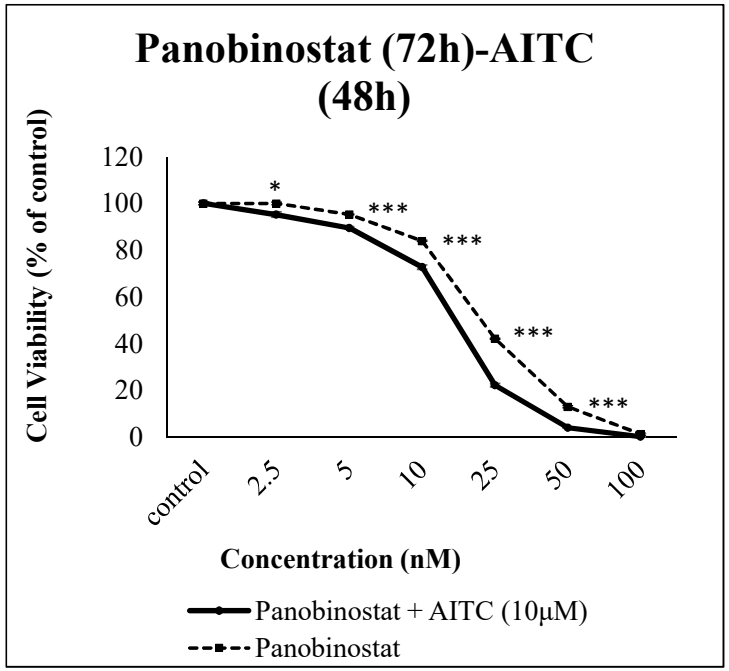
Inhibition Of HDAC Potentiates the Effect of AITC





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

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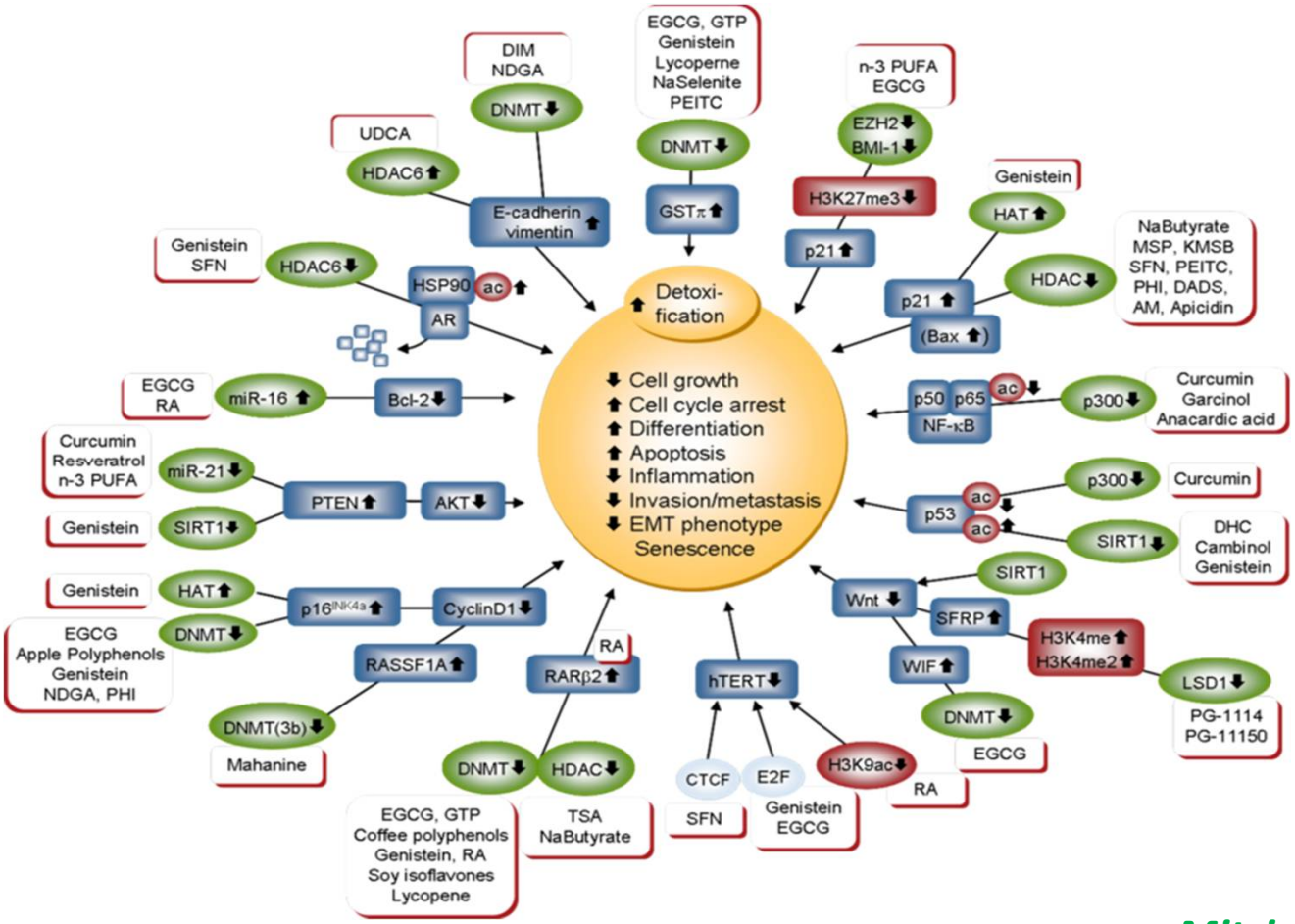
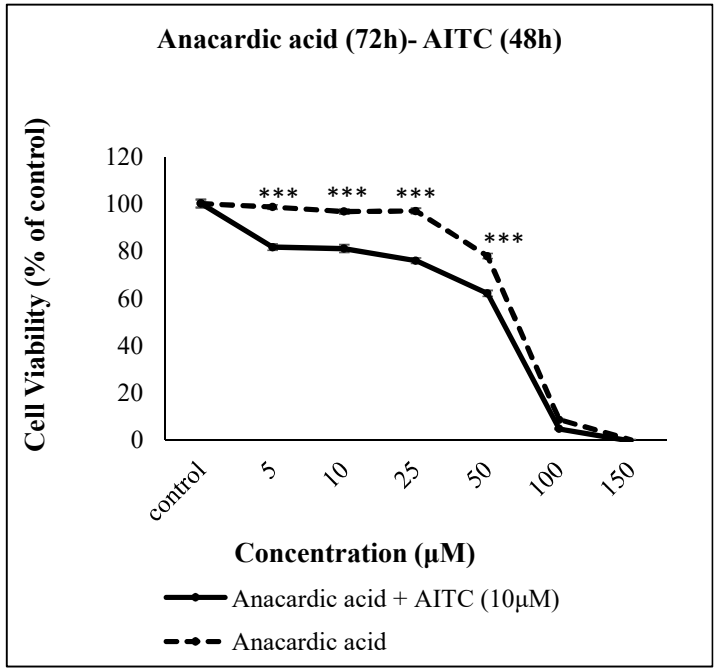
Inhibition Of HAT Potentiates the Effect of AITC





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

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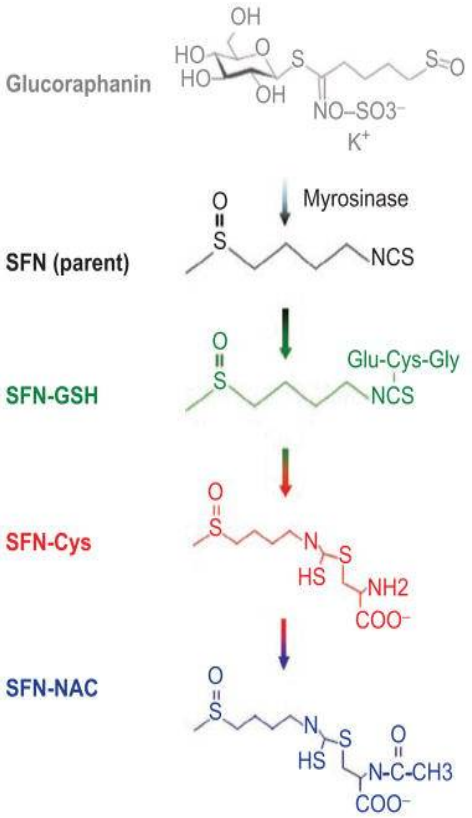
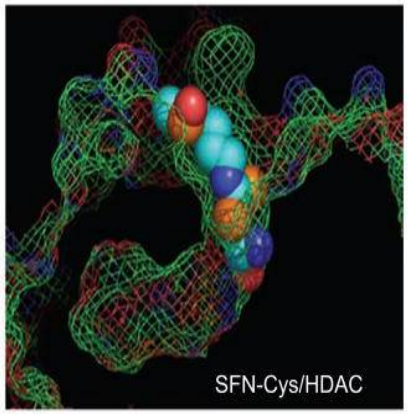
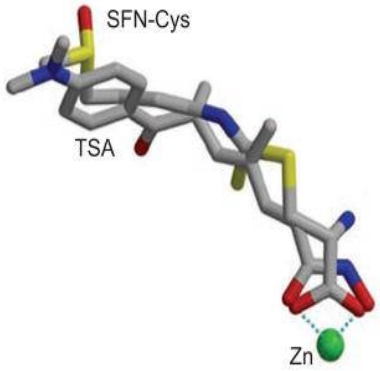
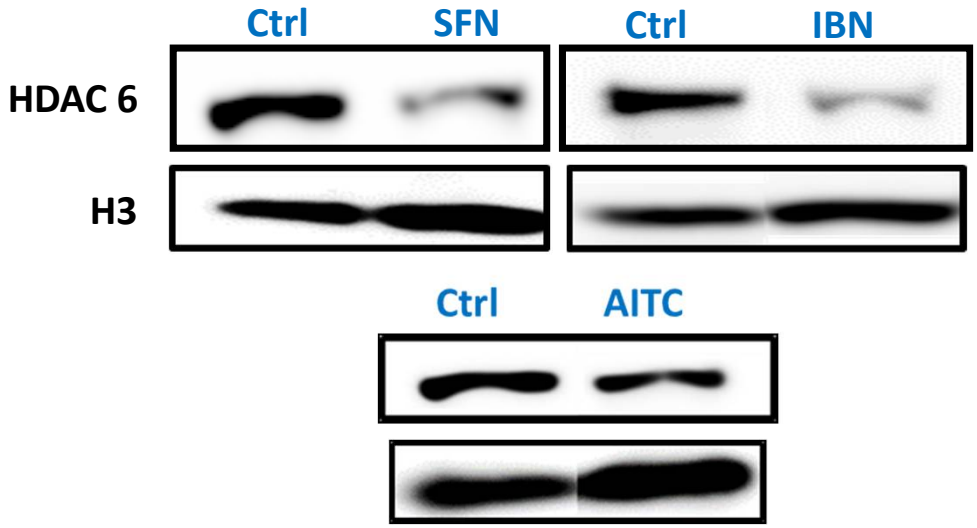
Epigenetic Therapy & HDACi







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

Rajendran et al. Crit Rev Biochem Mol Biol, 46(3): 181-199, 2011

Mitsiogianni et al. Eur J Nutr, 59(2): 557-669, 2020
Mitsiogianni et al. Eur J Nutr, 2020 (in press)

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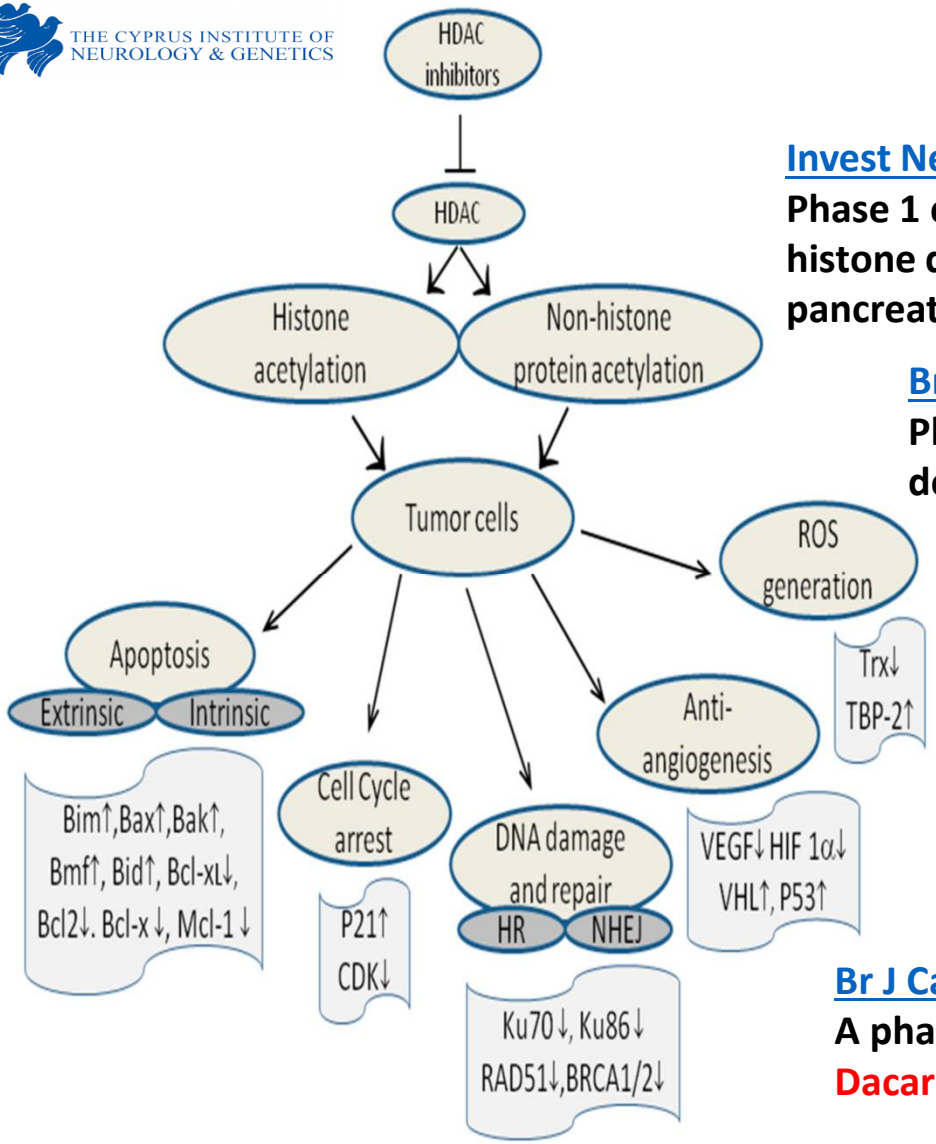
Epigenetic Therapy & HDACi



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

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.

Br J Cancer. 2009 Oct 6;101(7):1044-1050.
Phase I trial of Vorinostat and Doxorubicin in solid tumours: histone deacetylase 2 expression as a predictive marker.

Melanoma Res. 2008 Aug;18(4):274-27
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.

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Ms Venetia Tragkola

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Dr Trafalis

Northumbria University NEWCASTLE

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Prof Zoumpourlis

Newcastle University

Dr Bronowska

Nebraska University of Nebraska Lincoln

Dr Franco

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DALHOUSIE UNIVERSITY

Prof Rupasinghe

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Prof Ulukaya

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