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Epigenetic Events and Radiation Exposure

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Overview of the Talk

- What is Epigenetics?
- Major types of epigenetic events
- Evidence of involvement of epigenetic events in disease phenotypes
- Ionizing radiation (IR) induced diseases
- Evidence of IR-mediated effects involved with epigenetic changes
- Can epigenetic changes be used as a therapy for radiation damage?
- Challenges and possible suggestions

What is Epigenetics?

- Epigenetics is broadly defined as heritable changes in gene expression and its potential that do not necessarily involve changes in DNA sequence
- Epigenetic changes can occur mitotic as well as meiotic stages of cellular reproduction
- Epigenetic programming during development is an innate property for most living organisms, but disruptions of normal developmental programming of epigenetic states, caused by exposure of insults can have severe health consequences

Major Types of Epigenetic Events

- Methylation of cytosine residues on DNA (catalytic addition of a methyl group to the 5th carbon position of a cytosine residue that is followed on the same strand by guanine, called CpG dinucleotide – found mostly in CpG island promoter regions of genes)
- Chromatin remodelling through post-translational modifications of histone tails (acetylation, ubiquitination, phosphorylation)
- Modification of microRNA (miRNA) expression (through mRNA degradation or translational inhibition)

Epigenetics and Environmental Health

- Establishment of somatic cell epigenetic patterns in mammals occurs early in fetal development
- Re-establishment of appropriate epigenetic patterns is subsequent to genome-wide demethylation following fertilization, required for allowing totipotency and pluripotency dynamics (Morgan et al., 2005)
- Embryonic and fetal development represent a critical period during which nutrient availability as well as environmental stressors, including toxicant exposures, have great potential to affect the epigenetic reprogramming and patterning phenomena
- These have implications not only for proper development, but even for life-long conditioning and health (Li, 2002; Gluckman et al., 2008)

Toxicants/Factors influencing Epigenetic Alterations

Alcohol	Asbestos	Arsenic
Benzene	Radiation	Aging

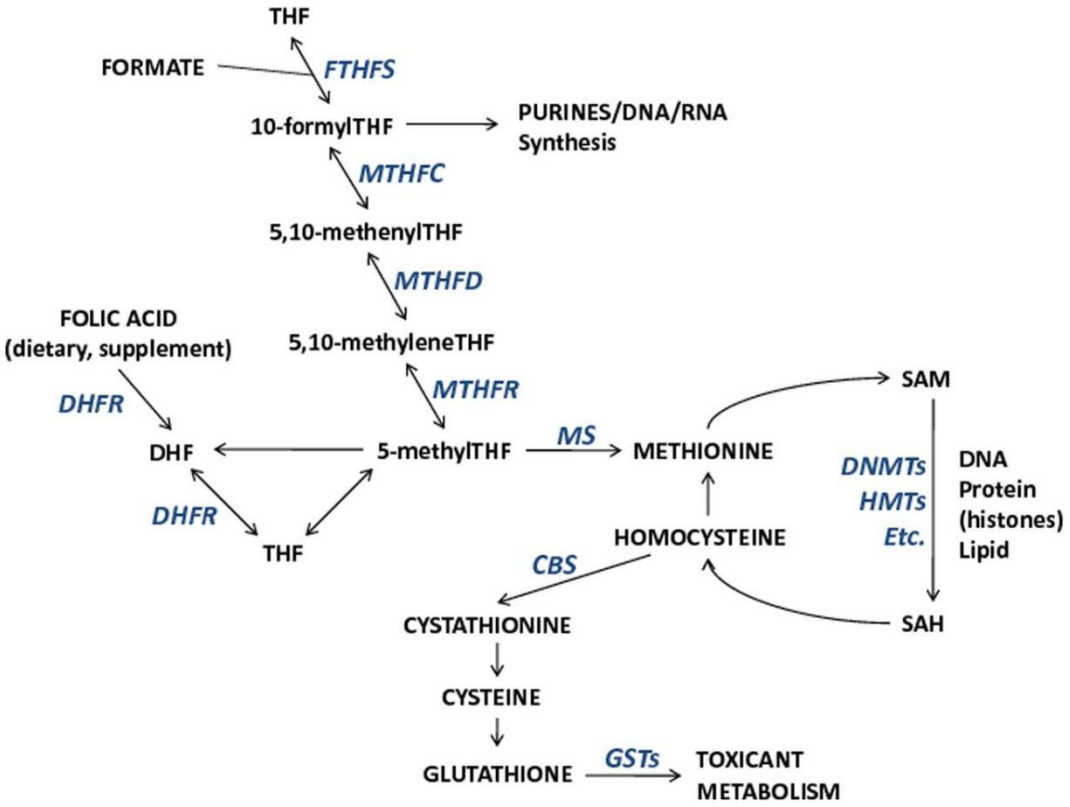
Other metals (e.g., Nickel, Cadmium, Chromium)

Endocrine disruptors (e.g., DES, BPA)

Particulates and Air pollution

Metabolic Components in One-Carbon Metabolism Model

(Source: Christensen and Marsit, 2011)



Enzymes that participate in metabolic reactions are italicized in blue. Metabolic Components: THF = tetrahydrofolate; DHF = dihydrofolate; SAM = S-adenosyl-methionine; SAH = S-adenosyl-homocysteine

Genome Function Beyond Genes

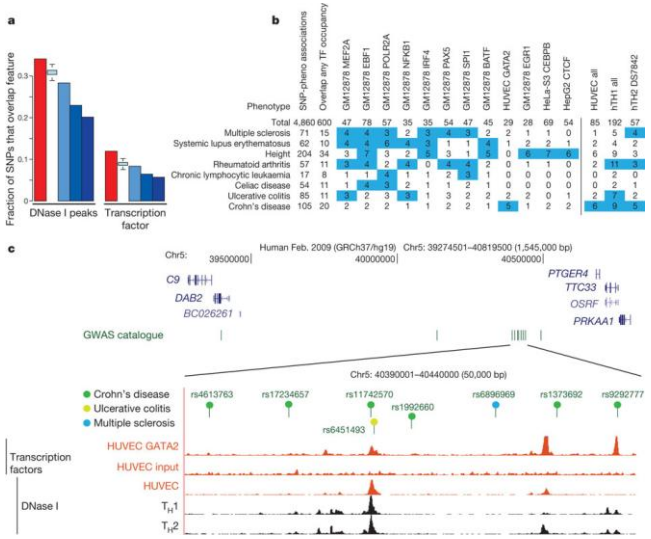
(Source: ENCODE Consortium 2012 Nature 489:57-74 and other papers on the same issue of Sept. 6, 2012)

- Over 80% of the human genome participates in at least one biochemical RNA- and/or chromatin-associated events in at least one cell type
- Much of the genome lies close to a regulatory event (95% within 8kb of a DNA-protein interaction site, and 99% within 1.7kb of at least one biochemical event)
- Their functions are asserted from evidence of negative selection in primate-specific elements and in those without mammalian constraints
- Enhancer-like features (in almost 400,000 regions) and promoter-like features (over 70,000 regions) are abundant in the genome
- Promoter functionality explains majority of variation of RNA expression
- Functional non-coding region of the genome is at least as large as the protein-coding regions of the genome
- Disease –associated SNPs (from GWAS data) are enriched within non-coding functional elements
- In many cases, disease phenotypes are associated with a specific cell-type or transcription factor

Together, these imply that genomic regions beyond genes are susceptible for epigenomic alterations

GWAS, ENCODE, AND DISEASES

Comparison of genome-wide-association-study-identified loci with ENCODE data.



I Dunham *et al. Nature* **000**, 1-18 (2012) doi:10.1038/nature11247

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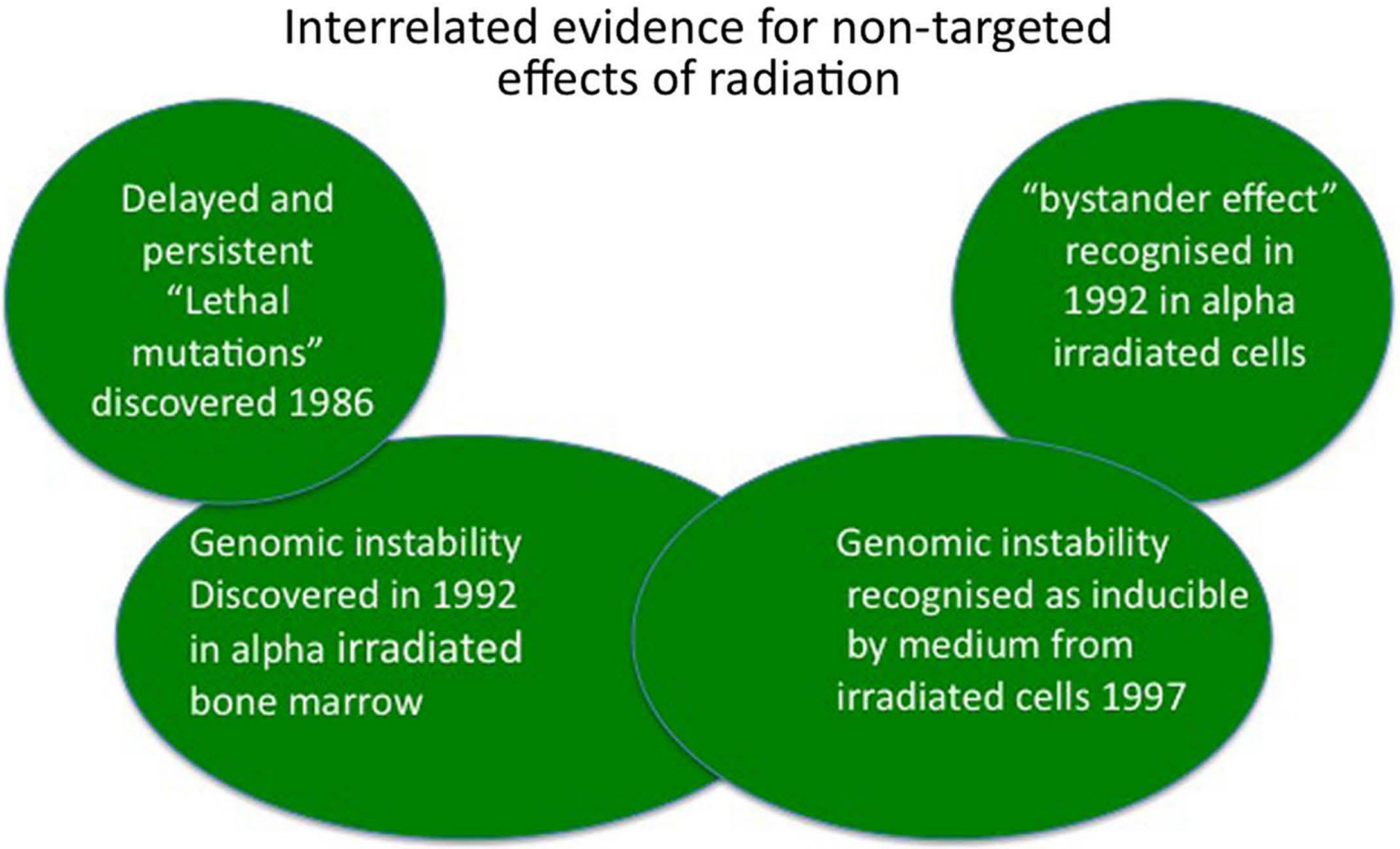
Radiation-Induced effects and Epigenetic Changes

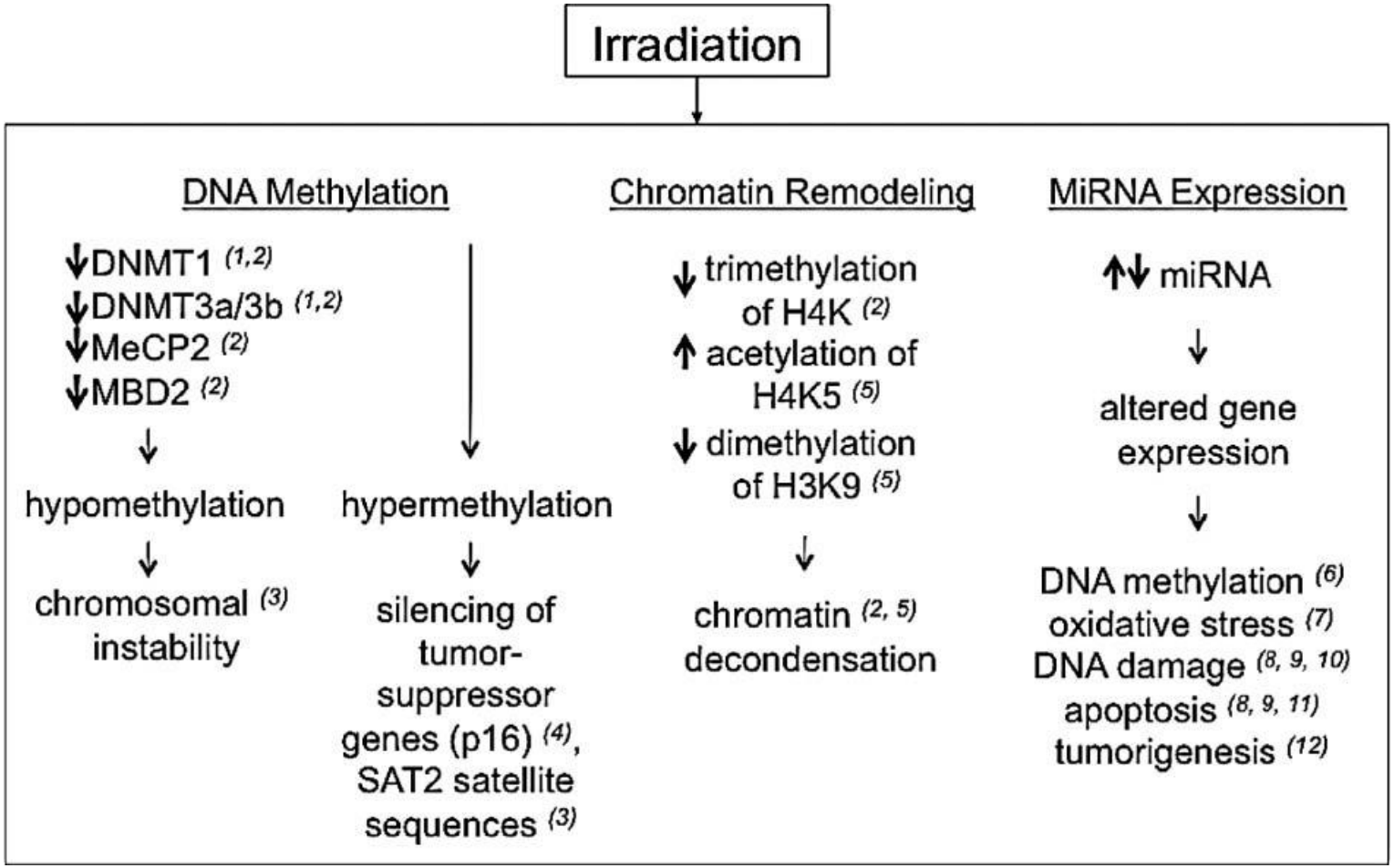
Initial Evidence of Non-targeted Radiation Effects

(Source: Mothersill and Seymour, Frontiers in Genetics, May 17, 2012)



Three Types of Epigenetic Changes are induced by Radiation

(Source: Aypar, Morgan, Baulch 2011, Int. J. Radiat. Bio., 87:179-191)



IR-Induced Bystander Effect and RIGI

- The event through which cells communicate radiation induced stress signals to unexposed cells is called the *Bystander Effect* of radiation.
- The phenomenon of propagation of radiation-induced damages across cellular divisions is described as *Radiation-Induced Genomic Instability* (RIGI); RIGI also manifests into increased rate of *de novo* mutational events in the progenies of irradiated cells.

(Definitions adapted from Ilnytskyy and Kovalchuk (2011) Mut. Res. 714:113-125)

Consequences of RIGI

- Delayed reproductive death (seen in X-irradiated Chinese hamster ovary cells; Chang and Little, 1991, Int J Radiat Biol. 60:483-96)
- Chromosomal aberrations (DNA double-strand breaks in Chinese hamster ovary cells leading to delayed reproductive death; Chang and Little, 1992, Radiat Res. 131:53-59)
- Increased apoptosis (Jamali and Trott, 1996, Int J Radiat Biol. 70:705-9)
- Micronuclei formation (dicentric chromosomes in surviving V79 cells after X-irradiation; Jamali and Trott, 1996, Int J Radiat Biol. 70:705-9)
- Changes in gene expression (Transcriptional responses of p53R2 in human lymphoblastic cells; Tsai et al. 2006, Oncogene 25:622-32)
- Aberrant DNA methylation (seen in cultured human keratinocytes; Kaup et al. 2006, Mutat Res. 597:87-97)

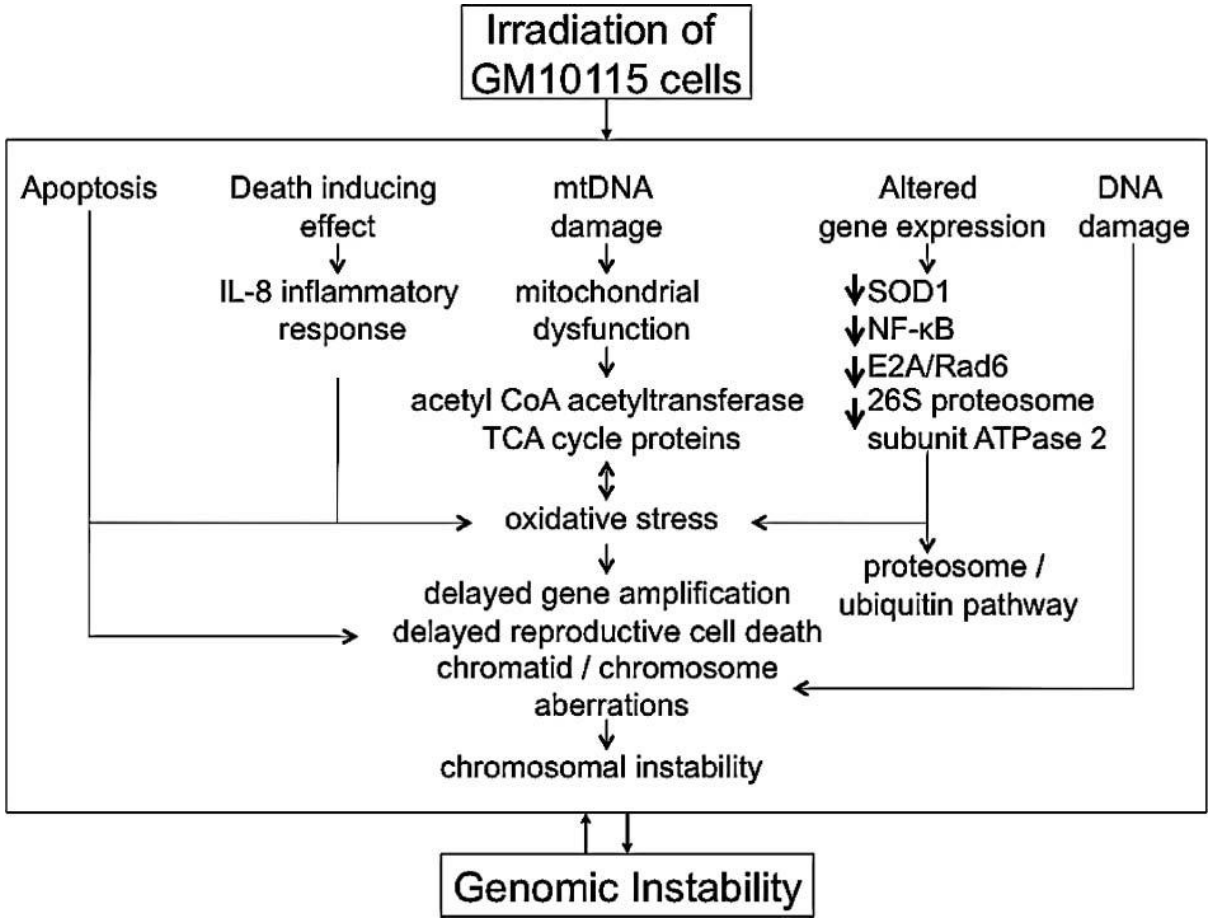
Comparison of Endpoints of Damage in Directed Irradiated Cells, Bystander Cells, and Progeny of Directly Irradiated Cells

(Adapted from Mothersill and Seymour (May 17, 2012), Frontiers in Genetics)

Endpoint	Directly irradiated cells	Radiation-induced bystander cells	Progeny of directly irradiated or bystander cells
Death	Reproductive death, apoptosis	Apoptosis and other forms of cell death	Delayed reproductive death, apoptosis
Protein induction	Repair Induction, checkpoint proteins	Induction of early response proteins	Persistent over-expression of stress proteins in progeny
Reactive oxygen species	Generation of free radicals	Oxidative stress	Persistent oxidative stress
Growth stimulation	Adaptive response	Proliferation and adaptive response	Adaptive response
Non-clonal persistent mutations	Chromosomal aberrations	Genomic instability(GI), lethal mutations	GI in progeny, Lethal mutatiions
Micronucleus assay	Increased Micronuclei	Cytogenetic effects, increased MN	Cytogenetic effects, increased MN
Carcinogenesis	Transformed foci	Transformed foci	Transformation and Cancer *in vivo*
Mitochondrial function	Aberrant	Aberrant	Aberrant
P53 function	Critical	Critical to response outcome	Critical to response outcome
Genotype dependency?	Yes	Yes	Yes

Phenotypes of Genomically Unstable Clones in Irradiated human-hamster cell line GM10115

(Source: Aypar, Morgan, Baulch 2011, Int. J. Radiat. Bio., 87:179-191)



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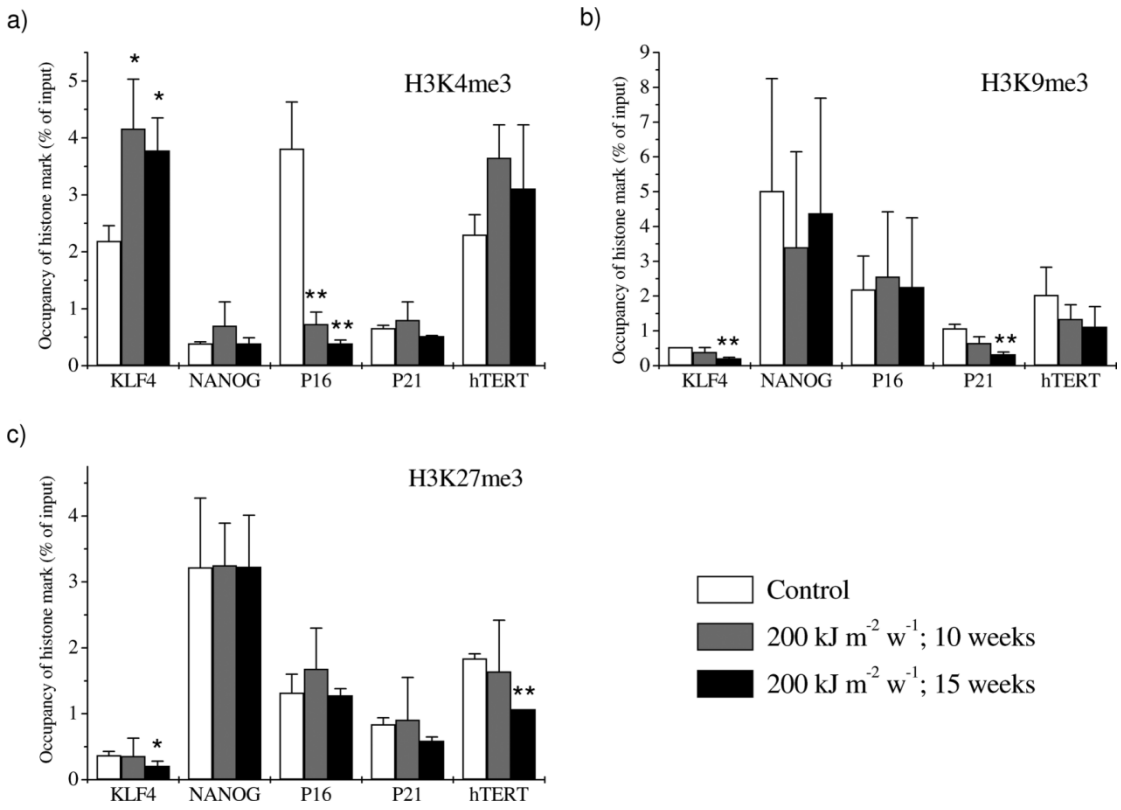
Histone Methylation pattern changes at the KLF4, P16INK4a, P21WAF1/CIP1 and hTERT promoter regions after chronic UVA-irradiation (200 kJm-2 week-1) of HaCaT human skin keratinocytes



Source: Chen et al. 2012, Photochem. Photobiol. Sci. 11:180-190

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Major Findings of Chen et al.'s (2012) Study of UVA-induced Epigenetic changes

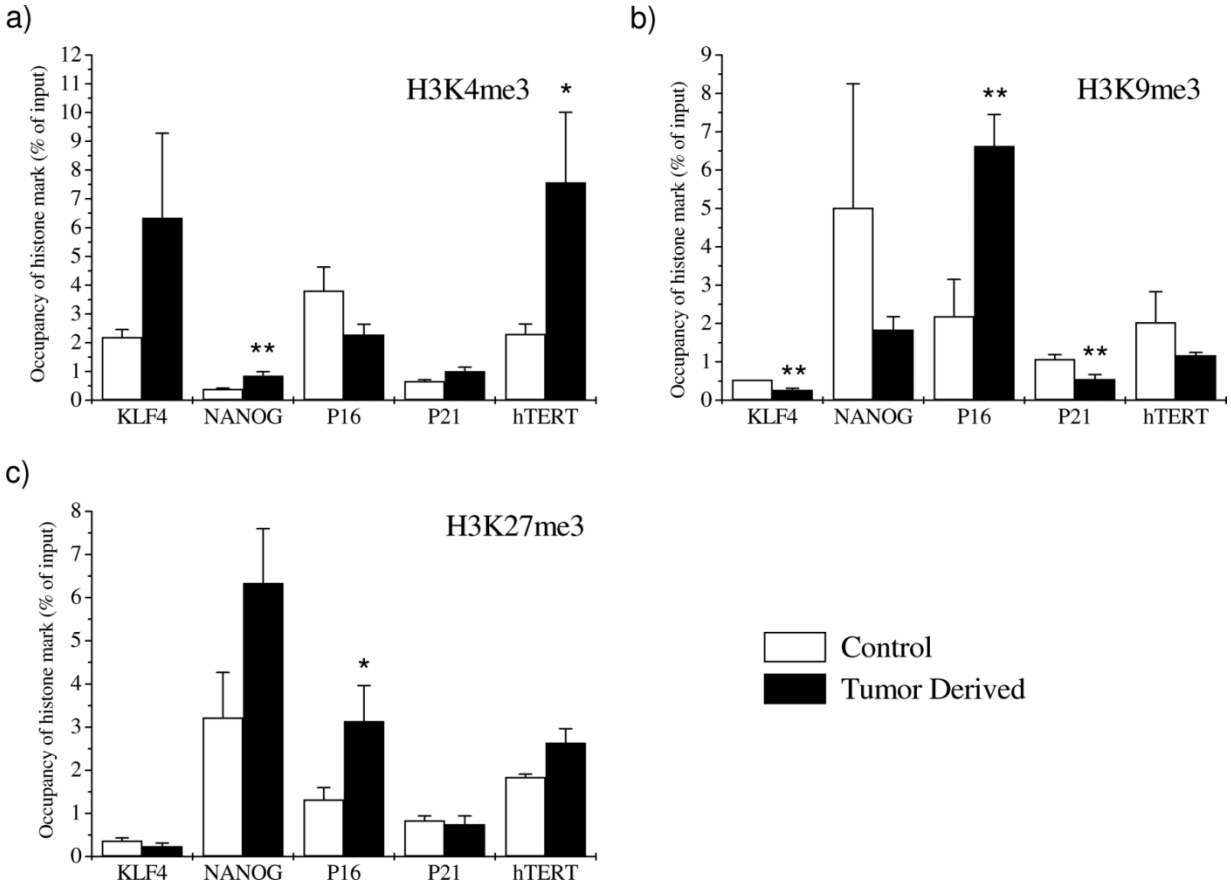
(a) Ten and fifteen weeks chronic UVA-irradiation caused pronounced reduction (decreased ~80% (10 weeks UVA) and ~90% (15 weeks UVA)) in the occupancy of the H3K4me3 mark at the promoter region of P16INK4a, as determined by ChIP-qPCR. An increase of H3K4me3 (~100%) was also obtained for KLF4 with 10 weeks and 15 weeks UVA-treatment.

(b) Fifteen weeks UVA-treatment caused reduction (~60%–~70%) of the H3K9me3 mark at the KLF4 and P21WAF1/CIP1 promoter regions.

(c) A slight reduction (~40%) of the H3K27me3 mark for KLF4 and hTERT was seen for the HaCaT cells 15 weeks treated with UVA.

Error bars indicate standard deviations. $N \geq 3$. *: $p < 0.05$; **: $p < 0.01$.

Histone methylation pattern changes at the KLF4, NANOG, P16INK4a, P21WAF1/CIP1 and hTERT promoter regions in tumor-derived cells (from 4 tumors of 4 mice) originating from chronically UVA-irradiated HaCaT skin keratinocytes (200 kJ m-2 week-1, 15 weeks)



Source: Chen et al. 2012, Photochem. Photobiol. Sci. 11:180-190

Major Findings of Chen et al.'s (2012) Study of UVA-induced Epigenetic changes in tumor-derived cells

(a) An increase of H3K4me3 was obtained for NANOG (100%) and hTERT (200%) in the tumor-derived cells.

(b) A striking increase (~200%) of the H3K9me3 mark at the P16INK4a promoter region was found in the tumor-derived cells. The occupancy of H3K9me3 at the KLF4 and P21WIF1/CIP1 promoters was slightly reduced (~50%).

(c) An increase of the H3K27me3 mark (100%) for P16INK4a was also obvious in the tumor-derived cells. Error bars indicate standard deviations

Error bars indicate standard deviations. $N \geq 3$. *: $p < 0.05$; **: $p < 0.01$.

Can Epigenetic Changes be Used as a Therapy for Radiation Damage?

Model for Effects of Bioactive Components for reversing epigenetic changes

(Source: Katiyer et al. Photochemistry and Photobiology, 2011; doi:10.1111.j.1751-1097.2011.010120.X)

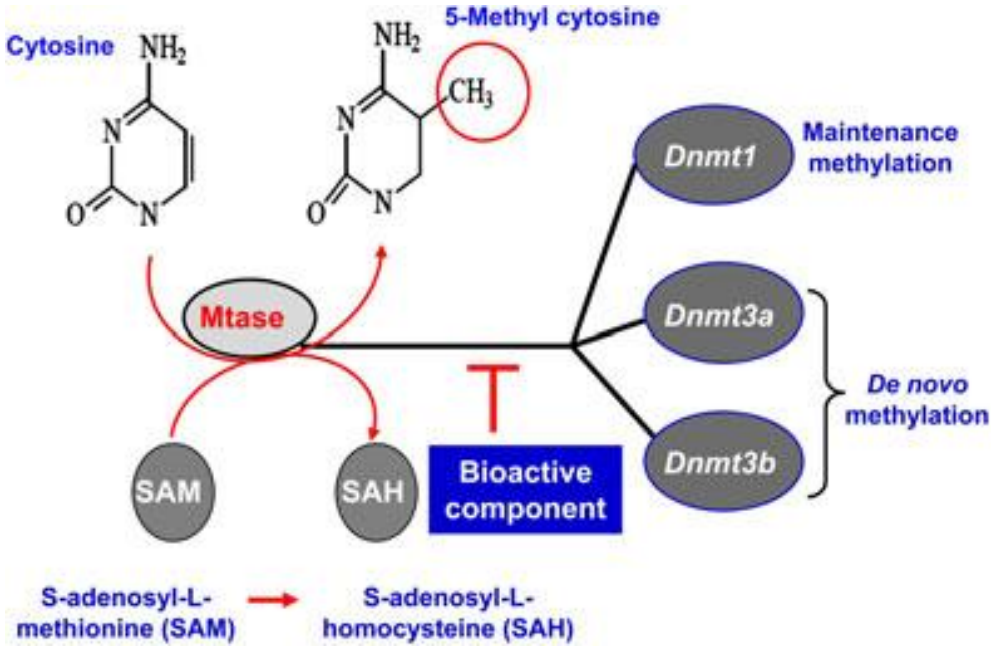


Diagram of conversion of cytosine into 5-methyl cytosine by the action of methyltransferase (Mtase). Different DNA methyltransferase enzymes play roles, such as Dnmt1, which has a role in normal maintenance of DNA methylation process while Dnmt3a and Dnmt3b are de novo methylation enzymes. The inhibitory effect of bioactive component on Dnmts activity will block/reduce DNA hypermethylation that lead to reversal of epigenetic alterations.

Examples of Dietary Biocomponents Related to Modulation of Epigenetic Alterations

- Folate (vitamin B_9) and other B vitamins (B_2, B_6, B_{12}), homocysteine, and methionine are important contributors to the maintenance of DNA integrity and DNA methylation (Christensen and Marsit, 2011)
- Dietary phytochemicals (e.g., green tea polyphenols, GTPs) have the ability to restore or reactivate the expression of the DNA hypermethylation-silenced genes, p16INK4a and Cip1/p21, in human skin cancer cells by downregulation of DNMT and HDAC activities Katiyar et al. 2011)
- Topical application of (-)-epigallocatechin-3-gallate (EGCG, 1 mg cm^{-2} skin area), a major and most active constituent of GTPs, blocks UVB-induced inflammatory responses, inhibits oxidative stress and photocarcinogenesis in mice (Mittal et al., 2003; Vayalill et al. 2003)

Challenges and Possible Solutions (if they exist) of Studying Epigenomics

Factors to Consider

Altered epigenetic programming and subsequent gene-expression patterns are influenced by:

- **Prenatal and early postnatal environmental factors**
- **Nutritional supplements**
- **Xenotoxic chemicals**
- **Adolescent and adult exposure due to life style and environmental factors**
- **Even Low-dose of radiation**

When did the Exposure Affected Epigenome?

If the stress exposure occurs during pregnancy of a mother (F_0), technically both F_1 (the embryo) and F_2 (its gamete) progenies have a chance to experience the stress exposure. The questions are:

- who are all that exposed (F_0, F_1, or F_2, or all?)
- What are their levels of expsoure?
- Is the effect (i.e., epigenetic alterations) truly transgenerational?

Other Challenging Questions

- Is transgenerational response caused equally by all stress factors?
- What developmental stage of an organism plays a major role in this response?
- Does the frequency of exposure affect the induction of a short-term (epigenetic) or long-term (genetic) response?
- How are the exposure levels to be determined when multi-generational entities are simultaneously exposed?

Solutions?

The candid answer is:

there is no solution available to these questions!

However, the ENCODE project and technologies being developed in that context suggest that:

- **Genomic assays can be developed to get signatures of exposure with detectable effects**
- ***In silico* cause-effect modeling may address determining simultaneous exposure levels in F_0, F_1, and F_2**
- **Genome annotation may help identifying regions affected by exposure and possibly phenotypes impacted by exposures**

Conclusions

- Epigenetic events are real, measurable, and their effects on health and disease are detectable
- All major categories of epigenetic alterations have possible health consequences during life time of individuals as well as in their progenies
- Timing of epigenetic stress exposures is critical for the severity of effects of epigenetic alterations
- Epigenetic alterations are reversible, and hence, understanding of the mechanism and stages of alterations may lead to therapeutic treatment of epigenetic ill-effects, including for the ones caused by radiation-induced epigenetic changes
- Effects of epigenetic changes go beyond the diseases that are induced by radiation exposure

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Thank you!

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