



EPIGENETICS AND SELECT BIOMARKERS OF ORGAN TOXICITY

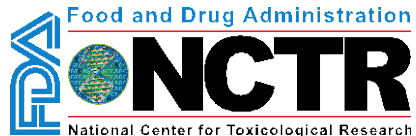
November 7, 2014

Igor Pogribny: “Investigating the Role of Epigenetics for Carcinogen Identification”.

Xi Yang: “Extracellular microRNA as Potential Biomarkers of Drug-Induced Liver Injury”.

James Fuscoe: “Global Functional Genomic and Epigenomic Changes in the Liver and Kidney during the Rat Life Span May Impact Susceptibility to Drugs and Disease”.

Beverly Lyn-Cook: “Epigenetic and Autoimmune Diseases: Potential New Targets for Therapeutic Intervention”.



INVESTIGATING THE ROLE OF EPIGENETICS FOR CARCINOGEN IDENTIFICATION

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Division of Biochemical Toxicology

NCTR, FDA

Epigenetics and Selected Biomarkers of Organ Toxicity

SAB, November 7, 2014

The views expressed in this presentation do not necessarily represent those of the
U.S. Food and Drug Administration

EPIGENETICS

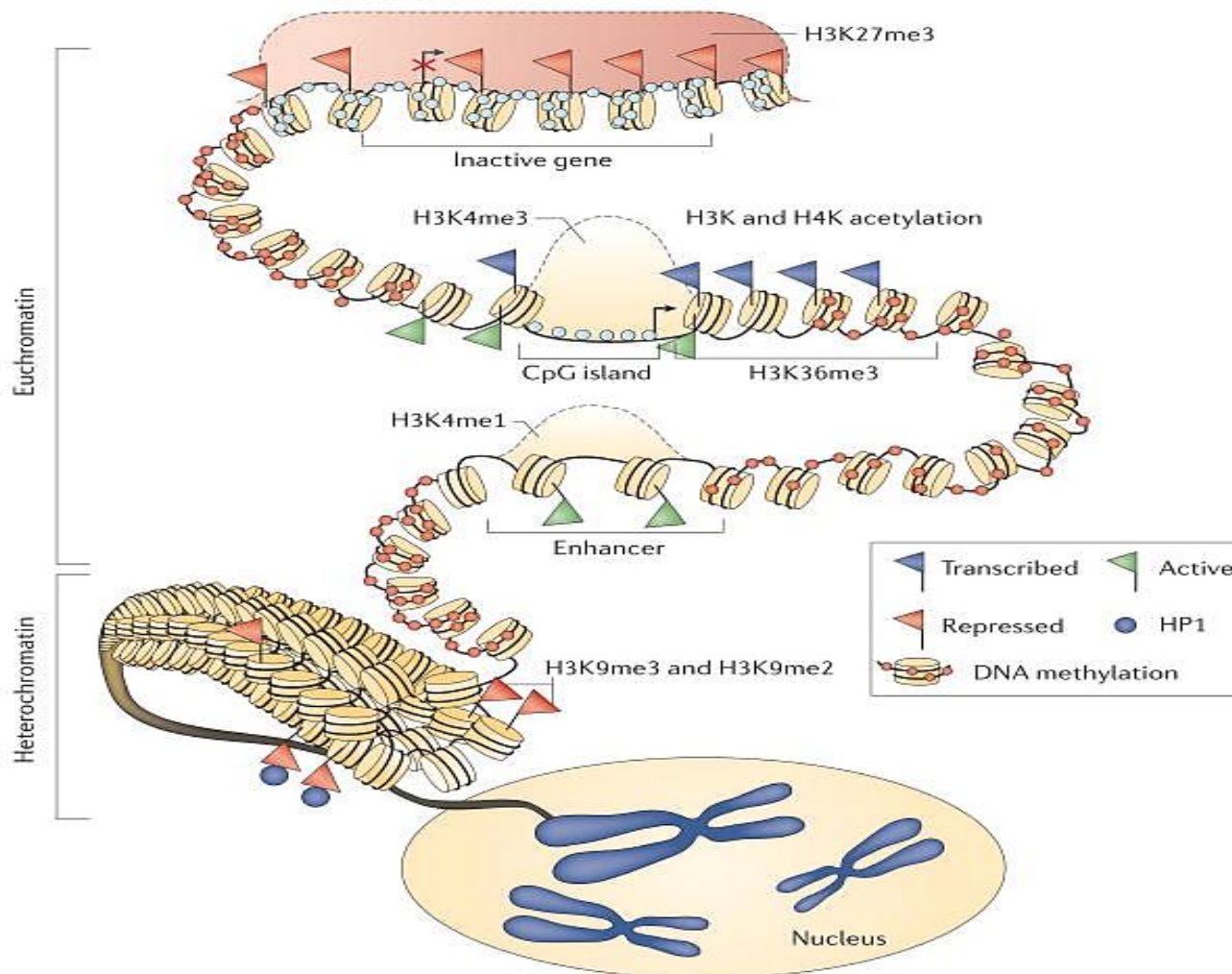
EPIGENETICS – heritable changes in gene expression mediated by methylation of DNA, modification of histone proteins, nucleosome positioning along DNA, or non-coding RNAs, that are not due to any alteration in the DNA sequence.

**Modifications at
the 5-position of
DNA-cytosine**

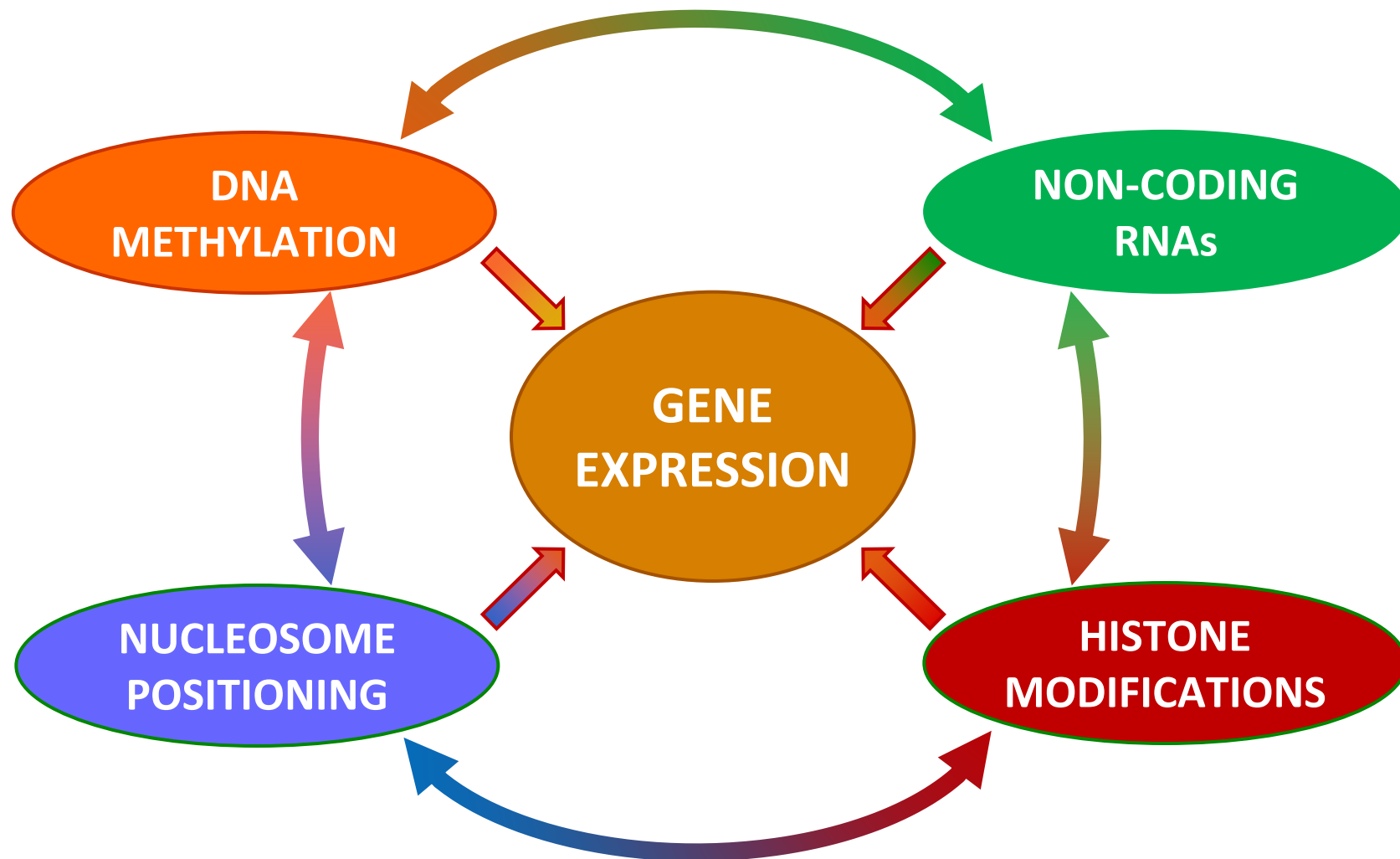
**Histone
modifications**

MicroRNAs

STRUCTURE OF THE EPIGENOME

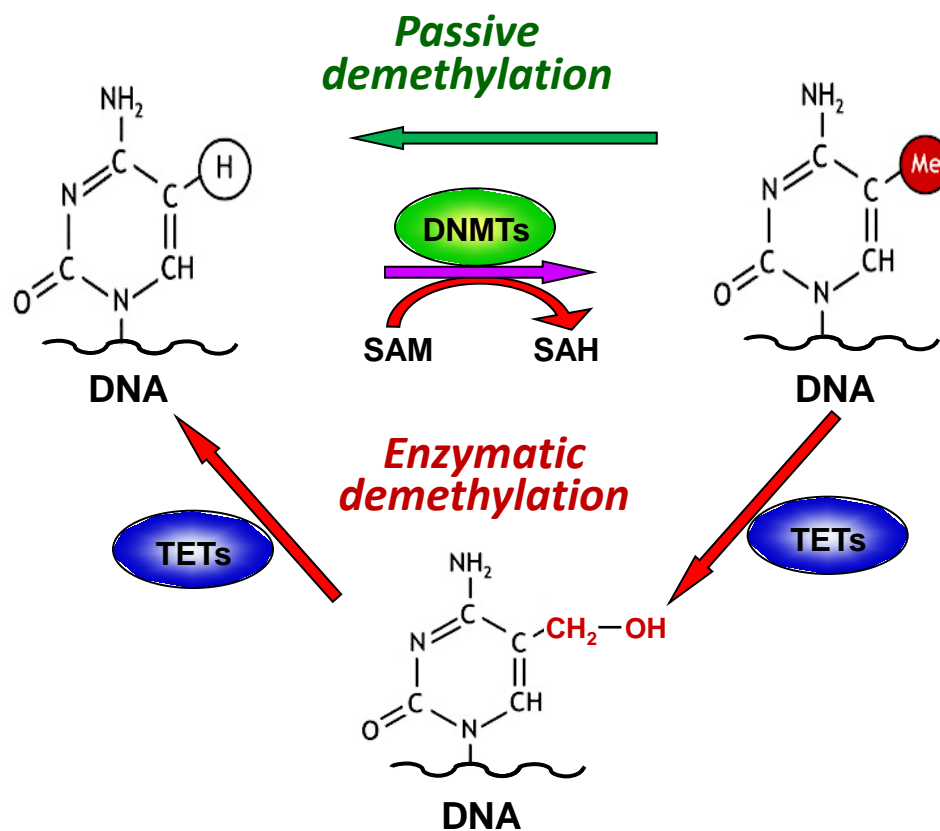


EPIGENETICS AND GENE EXPRESSION



DNA METHYLATION

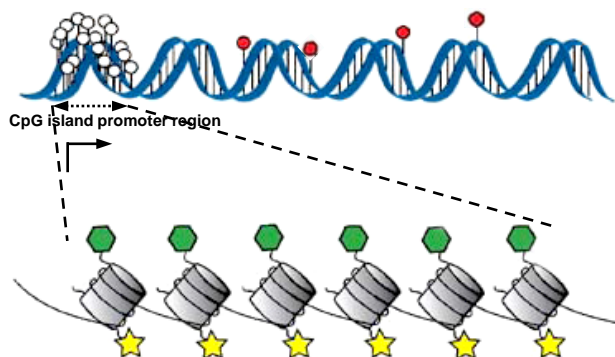
Cytosine DNA methylation is a covalent modification of DNA, in which a methyl group is transferred from S-adenosylmethionine (SAM) to the 5-position of cytosine by a family of cytosine (DNA)-methyltransferases



EPIGENOMIC ALTERATIONS IN CANCER

NORMAL CELL

DNA level



Chromatin level

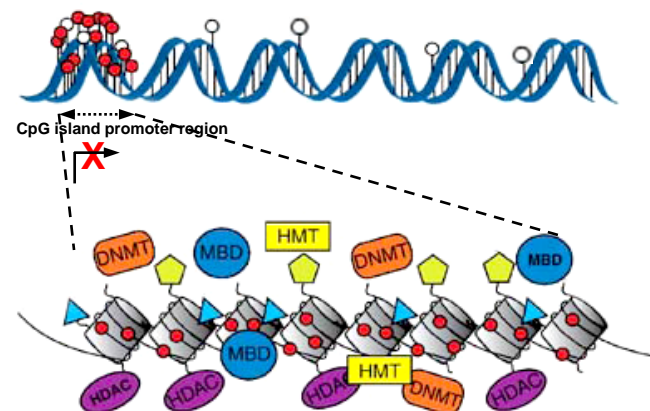
○ - unmethylated CpG sites

● - methylated CpG sites

- Global hypomethylation
- Promoter-specific hypermethylation
- Aberrant histone modification landscape
- Aberrant miRNA expression

CANCER CELL

DNA level

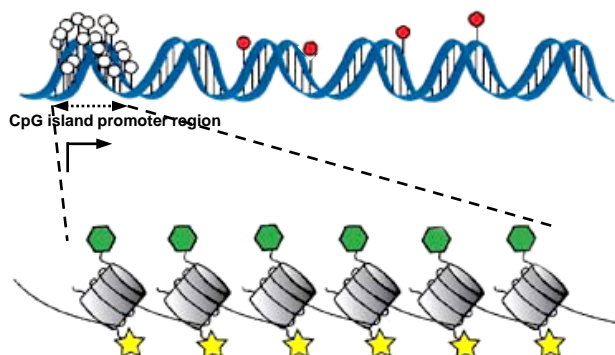


Chromatin level

EPIGENOMIC ALTERATIONS IN CANCER

NORMAL CELL

DNA level



Chromatin level

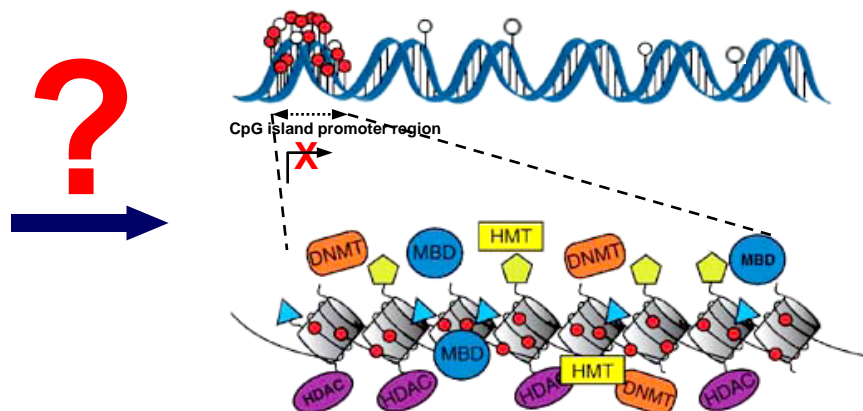
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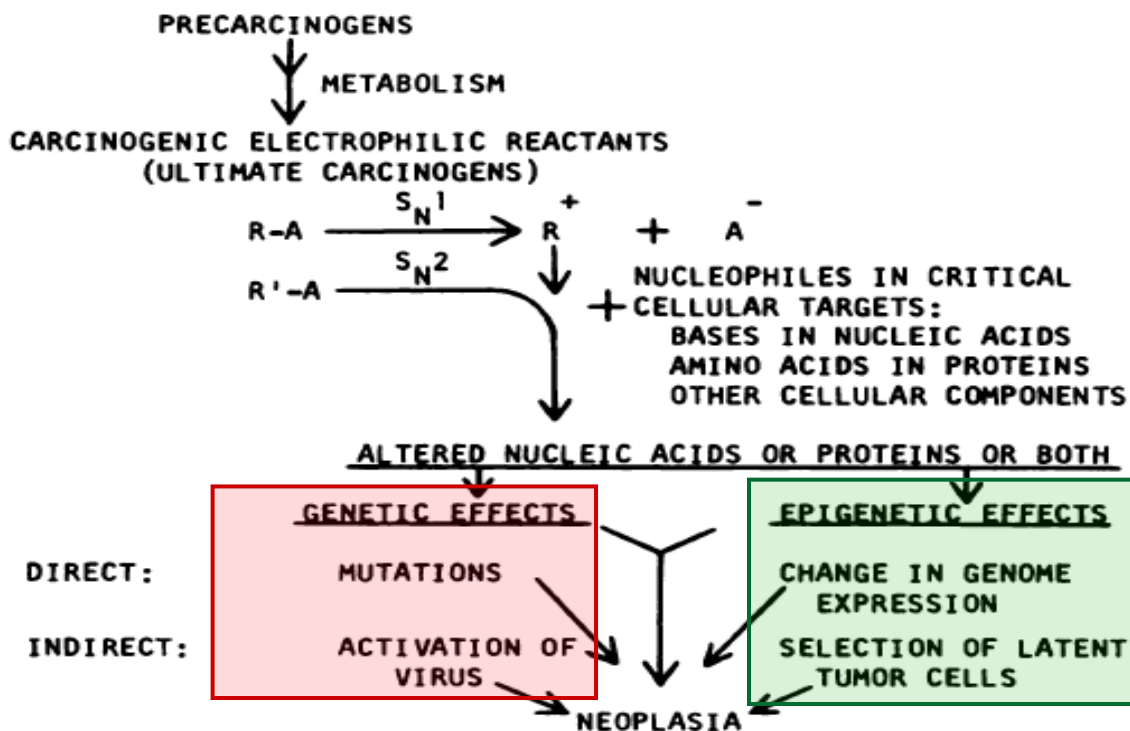
CANCER CELL

DNA level



Chromatin level

MECHANISMS OF CHEMICAL CARCINOGENESIS





EPIGENOMIC CARCINOGENICITY STUDIES IN NCTR

CARCINOGEN	MODE OF ACTION	SPECIES	EPIGENETIC CHANGES IN TARGET ORGAN	DIVISION
2-Acetylaminofluorene	Genotoxic	Rats	DNA methylation, histone modification, miRNA	DBT
Tamoxifen	Genotoxic	Rats	DNA methylation, histone modification, miRNA	DBT
1,3-Butadiene	Genotoxic	Mice	DNA methylation, histone modification	DBT
Riddelline	Genotoxic	Rats	DNA methylation, miRNA	DGMT
Furan	Non-Genotoxic	Rats	DNA methylation, histone modification, miRNA	DBT
Methyl donor-deficient diet	Non-Genotoxic	Rats, mice	DNA methylation, histone modification, miRNA	DBT
WY-14,643	Non-Genotoxic	Rats, mice	DNA methylation, histone modification, miRNA	DBT

DBT – Division of Biochemical Toxicology; DGMT – Division of Genetic and Molecular Toxicology



SIGNIFICANCE OF EPIGENETIC CHANGES IN CARCINOGENESIS

- **Epigenetic alterations are early events in the carcinogenic process and may be used as early indicators of exposure to carcinogens.**
- **Carcinogen-induced epigenetic alterations are not equal; there are “driver epigenetic changes” and “passenger epigenetic changes”.**
- **Carcinogen-induced epigenetic changes may be carcinogen-specific and tissue-specific.**
- **A single epigenetic alteration may be indicative of chemical carcinogenic potential.**

CHALLENGES IN CARCINOGENICITY ASSESSMENT

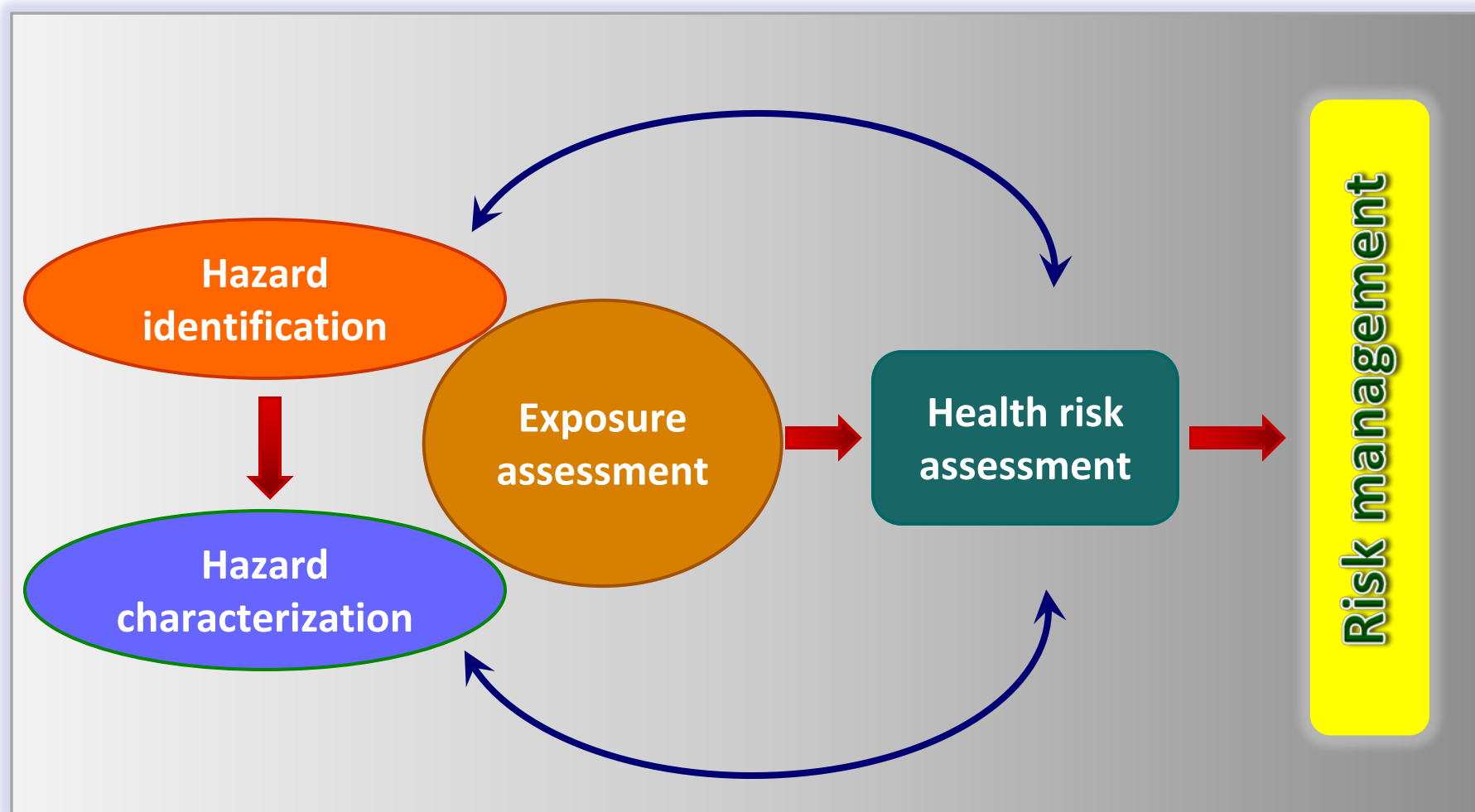
- **> 100,000 commercially manufactured chemicals are present in the human environment.**
- **The majority of chemicals have not been tested for their potential carcinogenicity.**
- **Many marketed drugs have not been tested according to current guidelines for carcinogenicity testing.**
- **No reliable test for rapid identification of non-genotoxic carcinogens.**



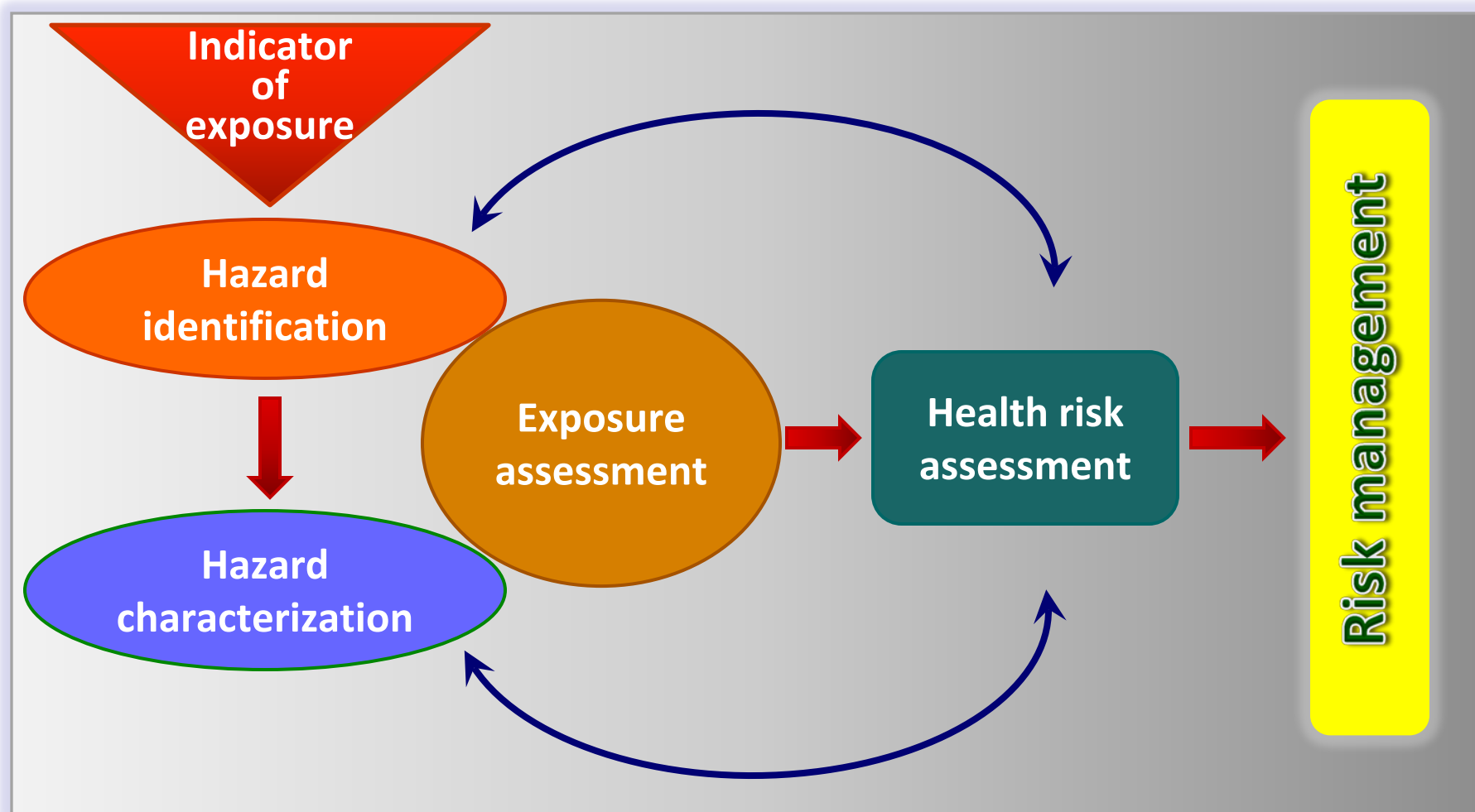
TWO-YEAR RODENT CARCINOGENICITY BIOASSAY

Purpose of study	Identify oncogenic effects for a major proportion of rodent normal lifespan.
Duration of study	Rats/mice 24 mo.
<u>Animals</u>	
Species/strain	Rats and mice, strains not specified.
Sexes	Males and females.
Age at initiation	Weanlings <6-8 weeks old.
Animal numbers	50/sex/group (≥25/sex/group desired at termination and initial number should be adjusted if toxicity is expected).
<u>Study design</u>	
No. dose groups	≥3 Dose groups.
Number of control groups	1 concurrent control group/sex; additional control group(s) if nutritional status may be altered by dose incorporation.
Duration and frequency of dosing	24 months - 7 days/week.

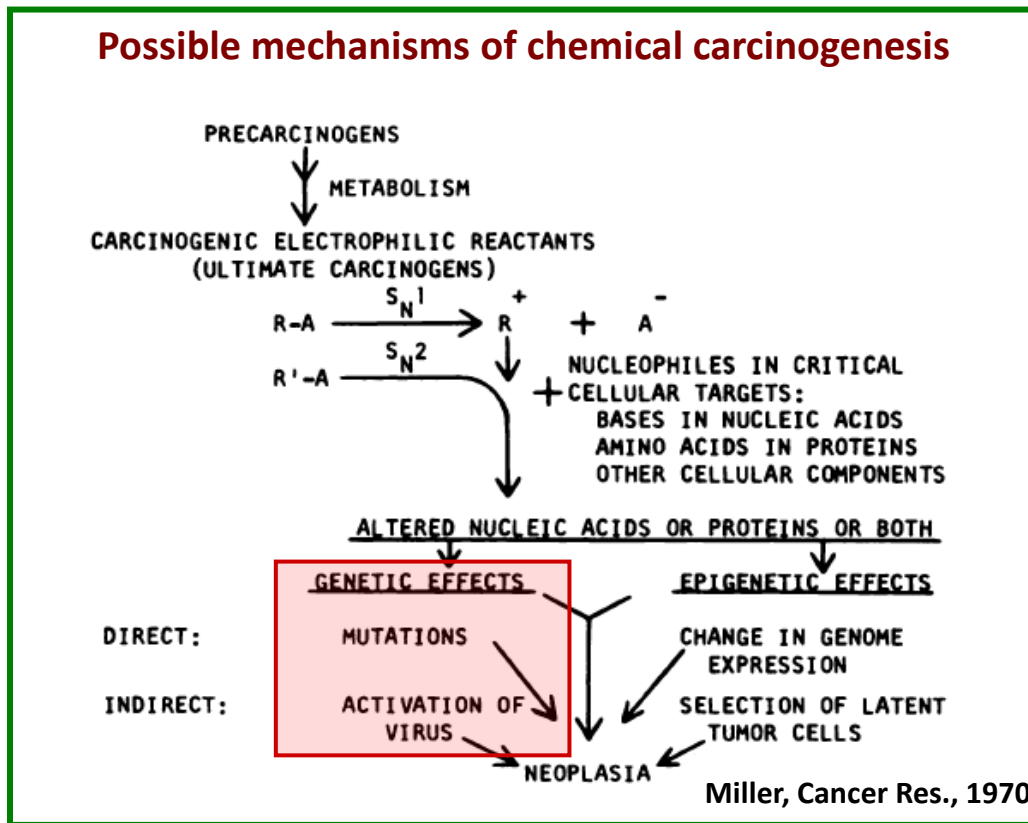
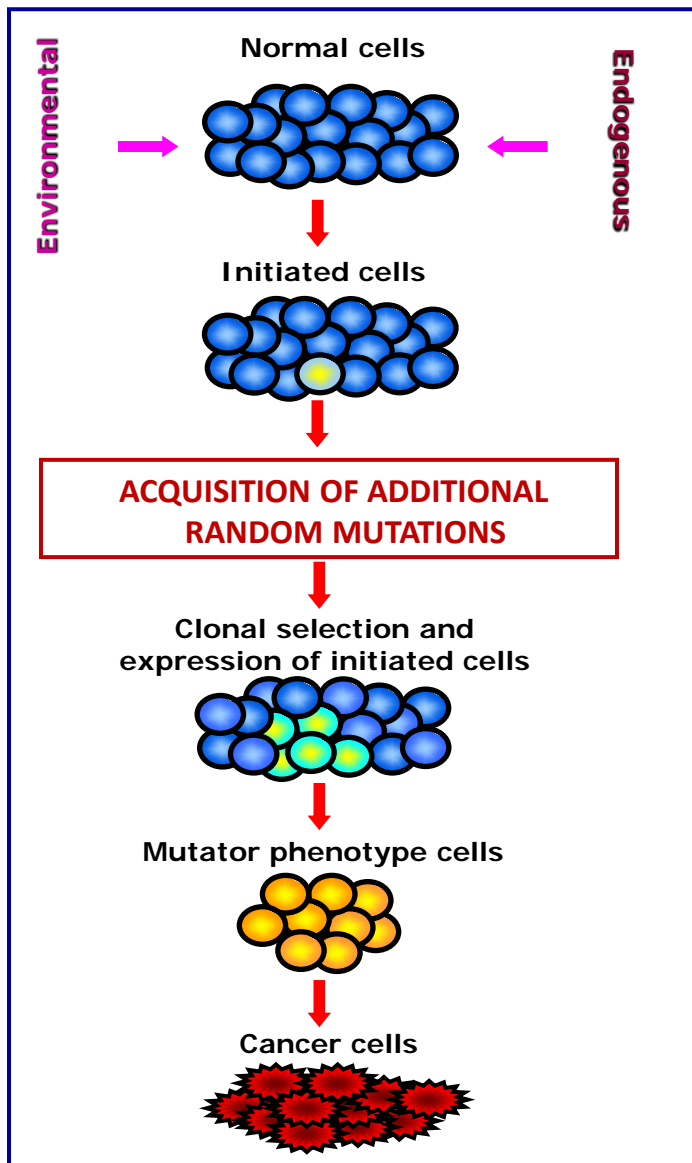
CARCINOGENICITY ASSESSMENT



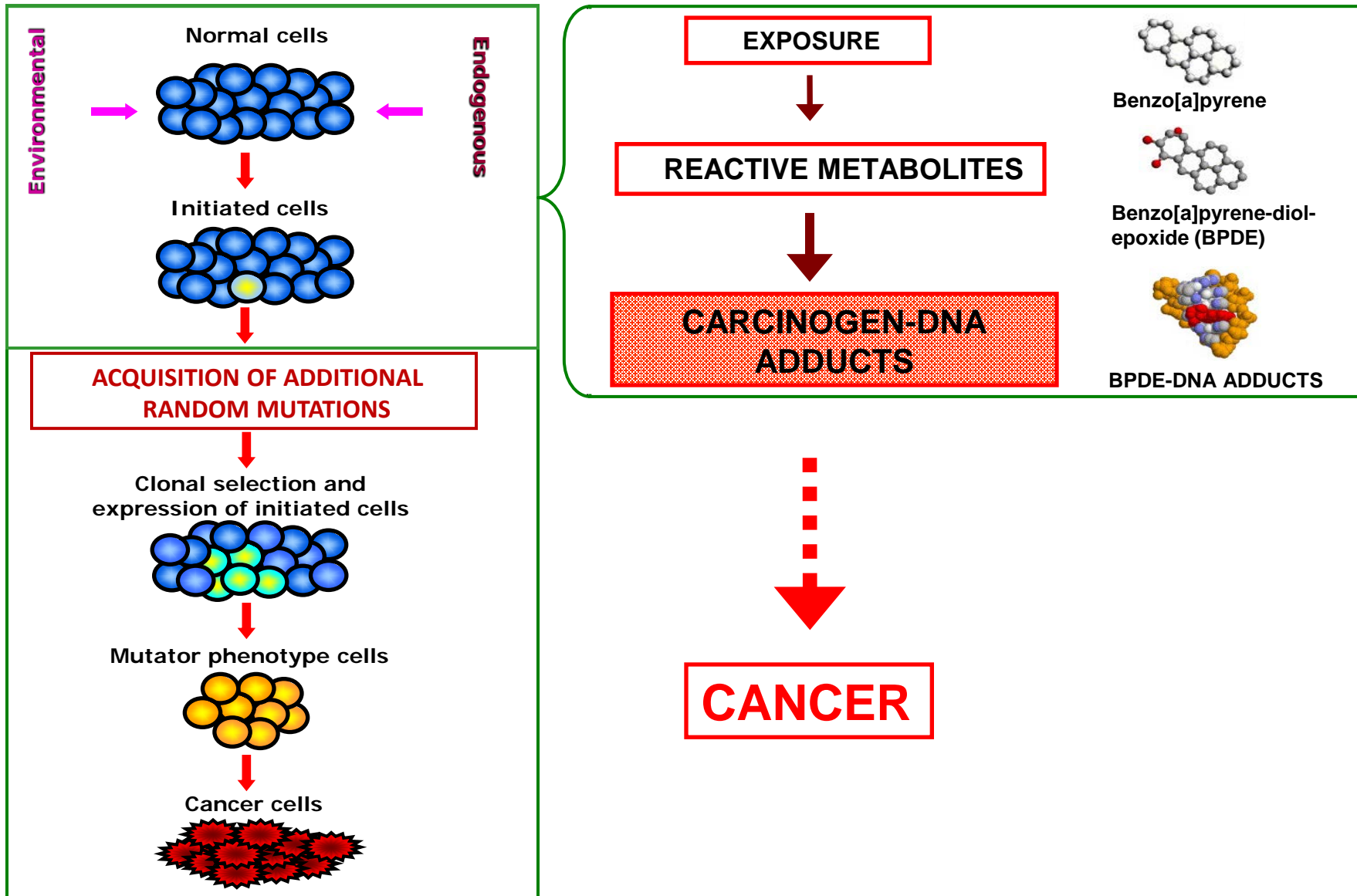
CARCINOGENICITY ASSESSMENT



CARCINOGEN-DNA ADDUCTS AND CANCER RISK ASSESSMENT



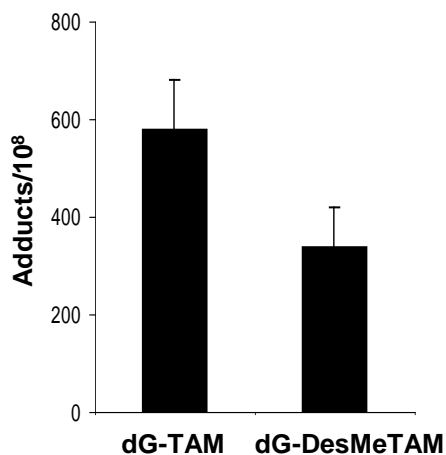
CARCINOGEN-DNA ADDUCTS AND CANCER RISK ASSESSMENT



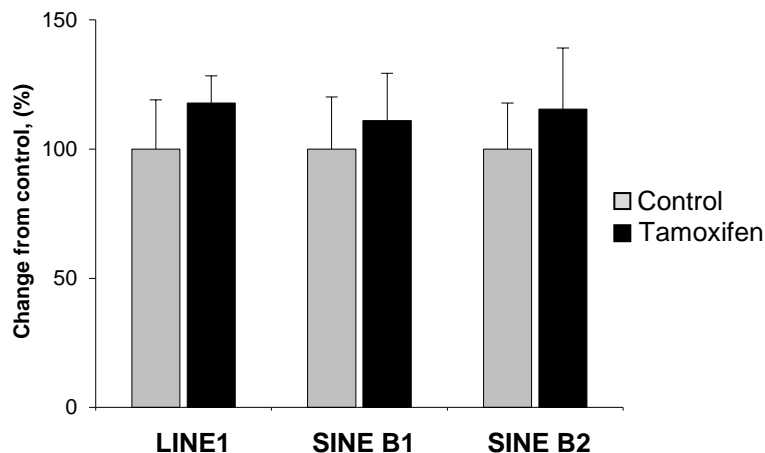
TAMOXIFEN-RODENT CARCINOGENICITY STUDIES

Mice

TAM-DNA adducts



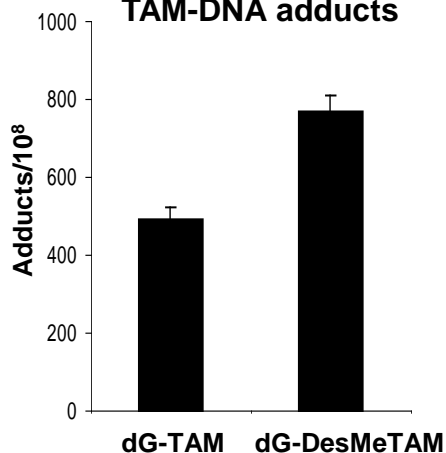
DNA repetitive elements methylation



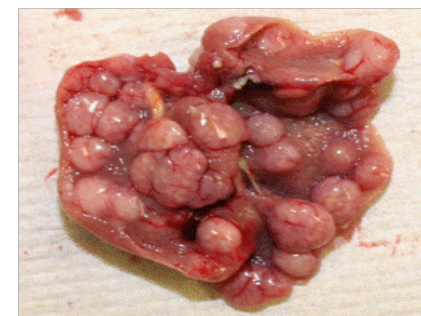
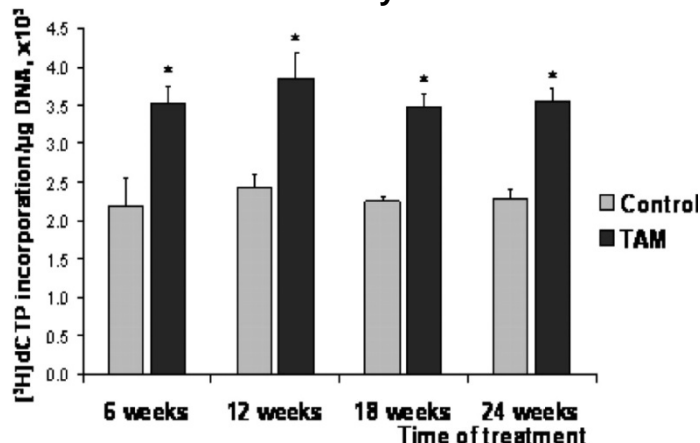
NO LIVER CANCER

Rats

TAM-DNA adducts

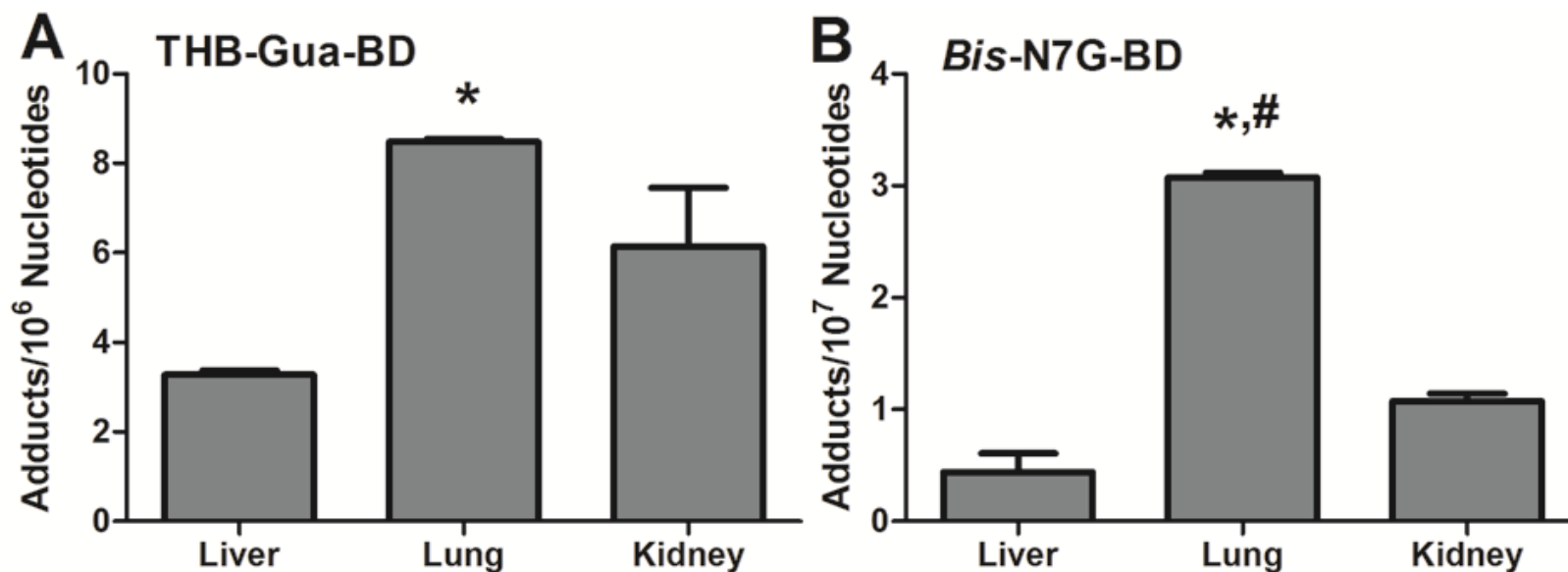


Global DNA methylation











LIVER CANCER

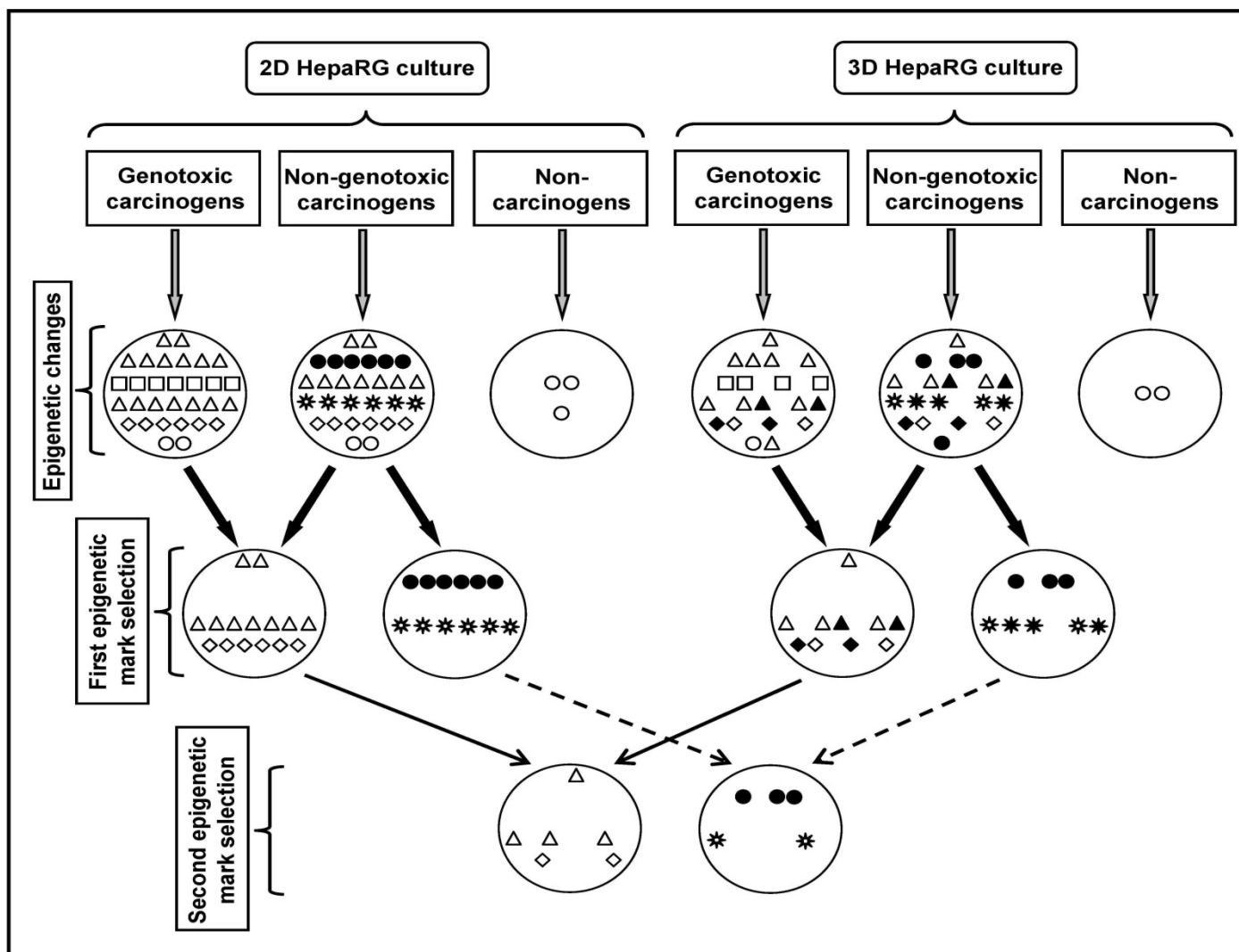
GENOTOXIC CHANGES INDUCED BY 1,3-BUTADIENE



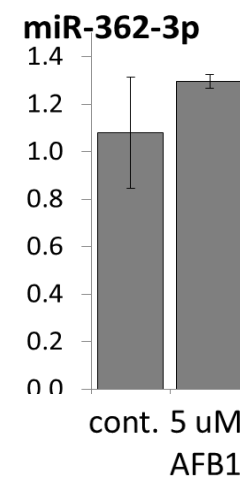
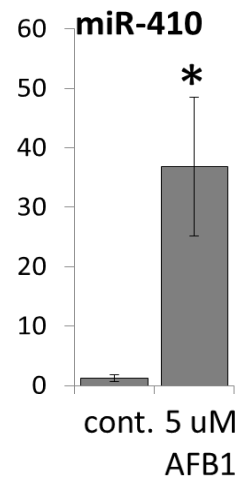
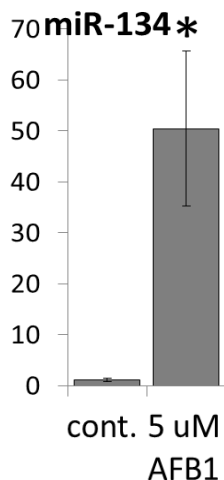
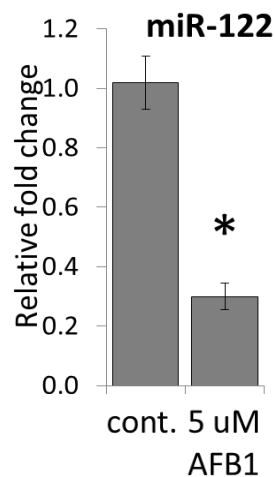
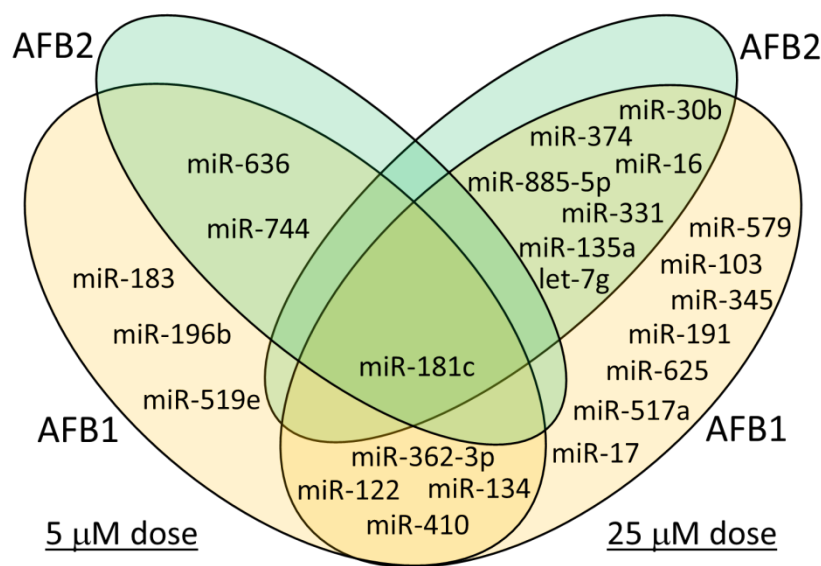
EPIGENETIC CHANGES INDUCED BY 1,3-BUTADIENE

	Liver	Lung	Kidney
DNA Damage	 <ul style="list-style-type: none"> • THB-Gua adducts • bis-N7-BD cross-links 	 <ul style="list-style-type: none"> • THB-Gua adducts • bis-N7-BD cross-links 	 <ul style="list-style-type: none"> • THB-Gua adducts • bis-N7-BD cross-links
DNA Methylation	 <ul style="list-style-type: none"> • SINEs B1 and B2 • Major and minor satellites 	 <ul style="list-style-type: none"> • SINE B2 • Major satellites • SINE B2 hydroxy-methylation 	
Histone Modifications	 <ul style="list-style-type: none"> • H3K27ac 	 <ul style="list-style-type: none"> • H3K56ac • H4K16ac 	 <ul style="list-style-type: none"> • H3K27me3 • H3K9me3 • H4K20me3

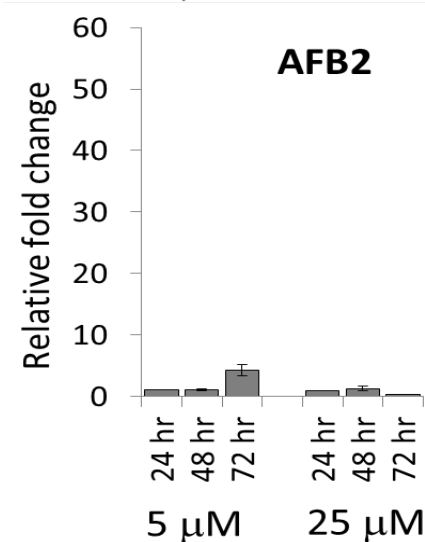
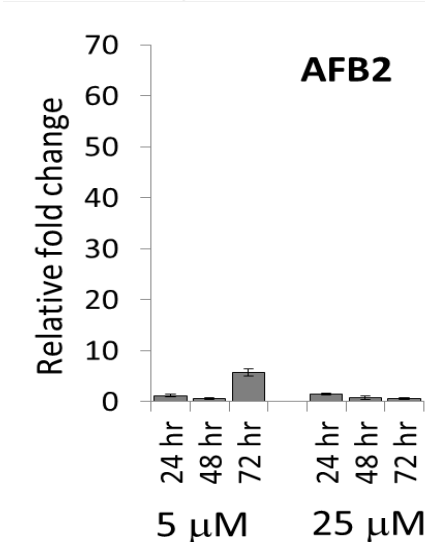
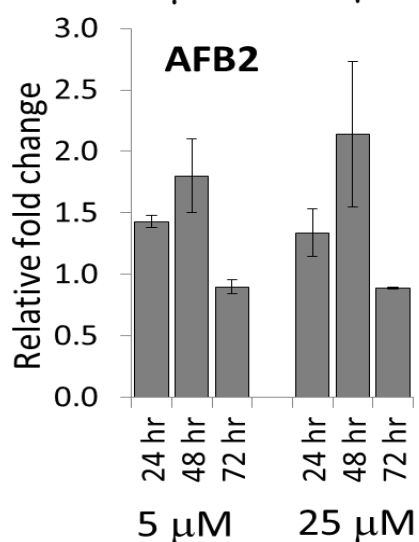
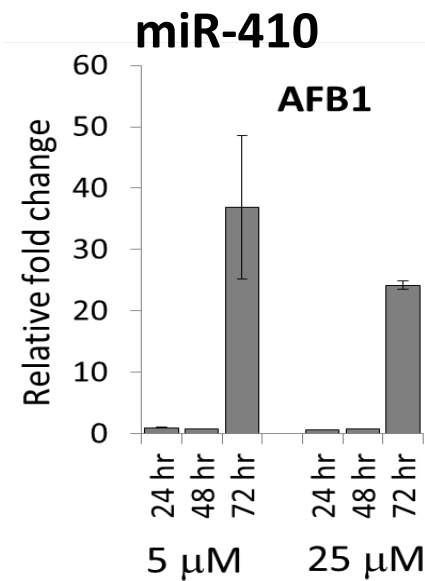
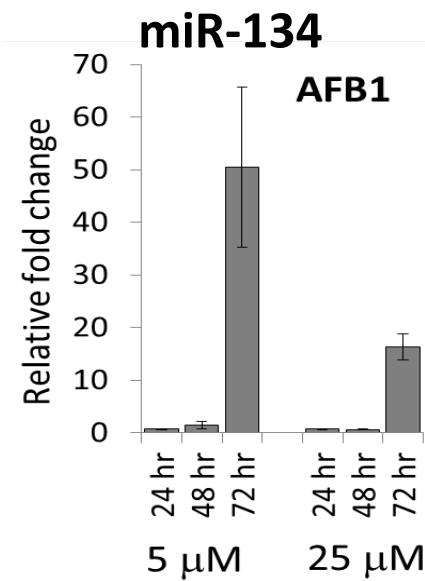
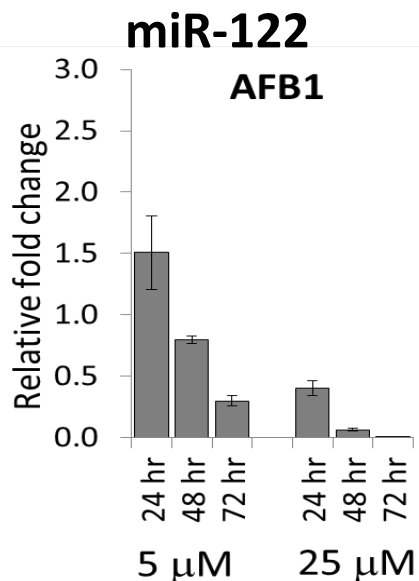
In Vitro EPIGENOMIC SCREENING MODEL



AFLATOXIN B₁-INDUCED miRNA ALTERATIONS *In Vitro*



AFLATOXIN B₁-INDUCED miRNA ALTERATIONS *In Vitro*



CONCLUSIONS

ADVANTAGES OF EPIGENETIC BIOMARKERS

- Early appearance.
- Stability.
- Target tissue specificity.
- Relevance to genotoxic and non-genotoxic carcinogens.
- Mechanistic value.
- Greater number of detectable epigenetic changes than genetic alterations.



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