Epigenomics

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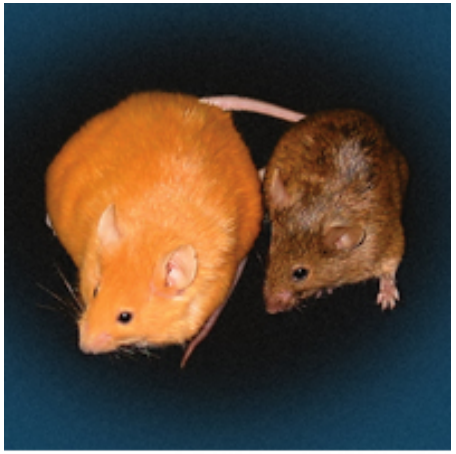


Randy Jirtle/Duke University

Outline

- 1. Epigenetics-definition and overview
- 2. DNA methylation/hydroxymethylation
- 3. Histone modifications
- 4. Nucleosome positioning
- 6. Methodology for assaying changes in DNA methylation and histone modifications
- 7. Epigenetic changes as biomarkers
- 8. Pharmacological targeting of epigenetic regulators

Goals

- List the epigenetic modifications and their importance in disease
- Understand the mechanisms that give rise to epigenetic alterations in disease
- Understand how epigenetic alterations can be used as biomarkers
- How epigenetic regulators can be targeted in disease

Epigenetics/epigenomics- a definition

- Any process that alters gene activity without changing the DNA sequence and leads to modifications that can be transmitted to daughter cells.
- Epigenomics: global study of epigenetic changes across the entire genome

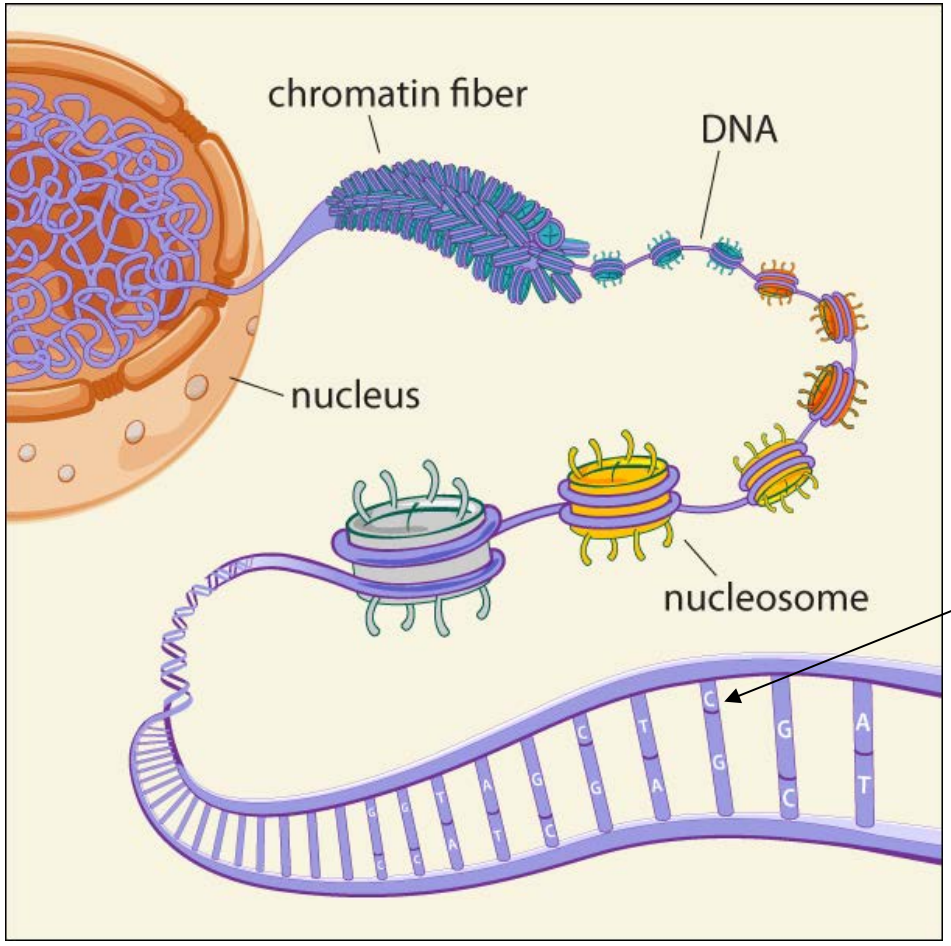
Epigenetics

- All cells in a multicellular organism have the same genetic material, however, not every gene of an organism is active in each cell at all times.

- Conrad Waddington (1905-1975) coined the term: “epigenetic landscape” to describe mechanisms that convert the genetic information into observable traits or phenotypes.

- Epigenetic gene expression patterns and the associated phenotypes, once established, may persist through cell divisions without the involvement of a change in DNA sequence.

DNA/Chromatin



Epigenetics on a molecular level

- Covalent modifications of cytosine bases and histones
- Positioning of nucleosomes

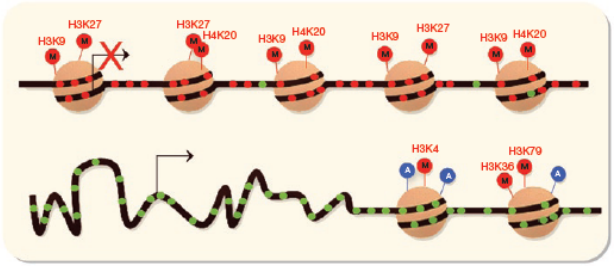


Figure 4 Nucleosome positioning patterns. Nucleosome positioning plays an important role in transcriptional regulation. Transcriptionally active gene promoters possess a nucleosome-free region at the 5′ and 3′ untranslated region, providing space for the assembly and disassembly of the transcription machinery. The loss of a nucleosome directly upstream of the TSS is also necessary for gene activation, whereas the occlusion of this position leads to transcription repression. DNA methylation regulates transcription, and thus interferes with nucleosome positioning. Methylated DNA seems to be associated with 'closed' chromatin domains, where DNA is condensed into strictly positioned nucleosomes, thereby impeding transcription. Conversely, unmethylated DNA is associated with 'opened' chromatin domains, which allow transcription.

Epigenetic modifiers

Gene expression

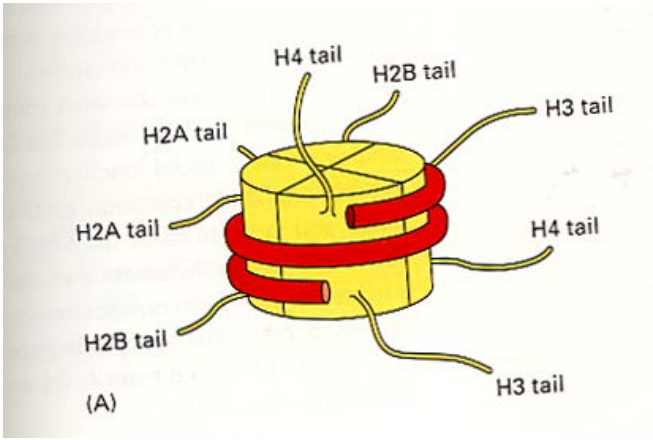
Non-coding RNA

mRNA

Nucleosome composition and Structure

Table 21-1 Properties of Histones from Calf Thymus

Histone	Composition	MW	Relative Molar Abundance
H1	Lys rich	21,000	1
H2A	Slightly Lys rich	14,500	2
H2B	Slightly Lys rich	13,700	2
H3	Arg rich	15,300	2
H4	Arg rich	11,300	2

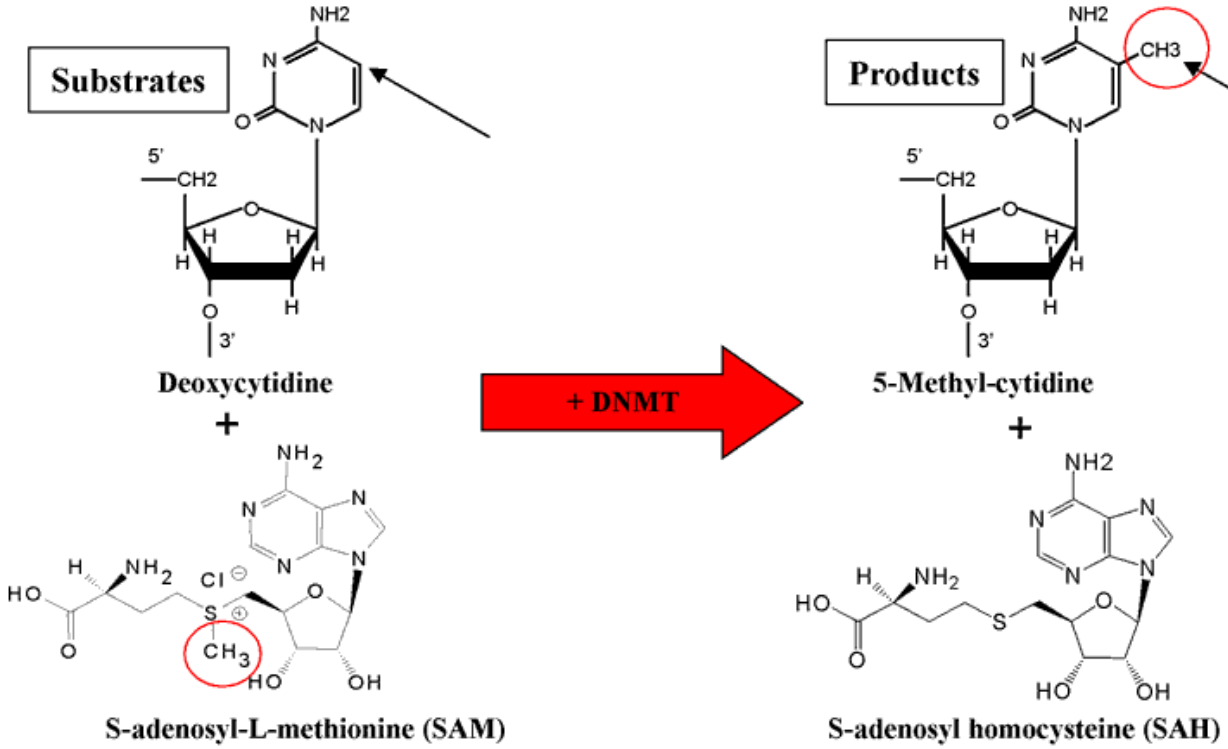


<u>Histone modifications</u>
Acetylation
Methylation
Phosphorylation
Ubiquitination
Sumoylation
Ribosylation

Covalent Modifications of DNA

Most widely studied epigenetic modification is cytosine methylation.

Methyl groups (red) in DNA (provided by Dr. Craig Cooney)



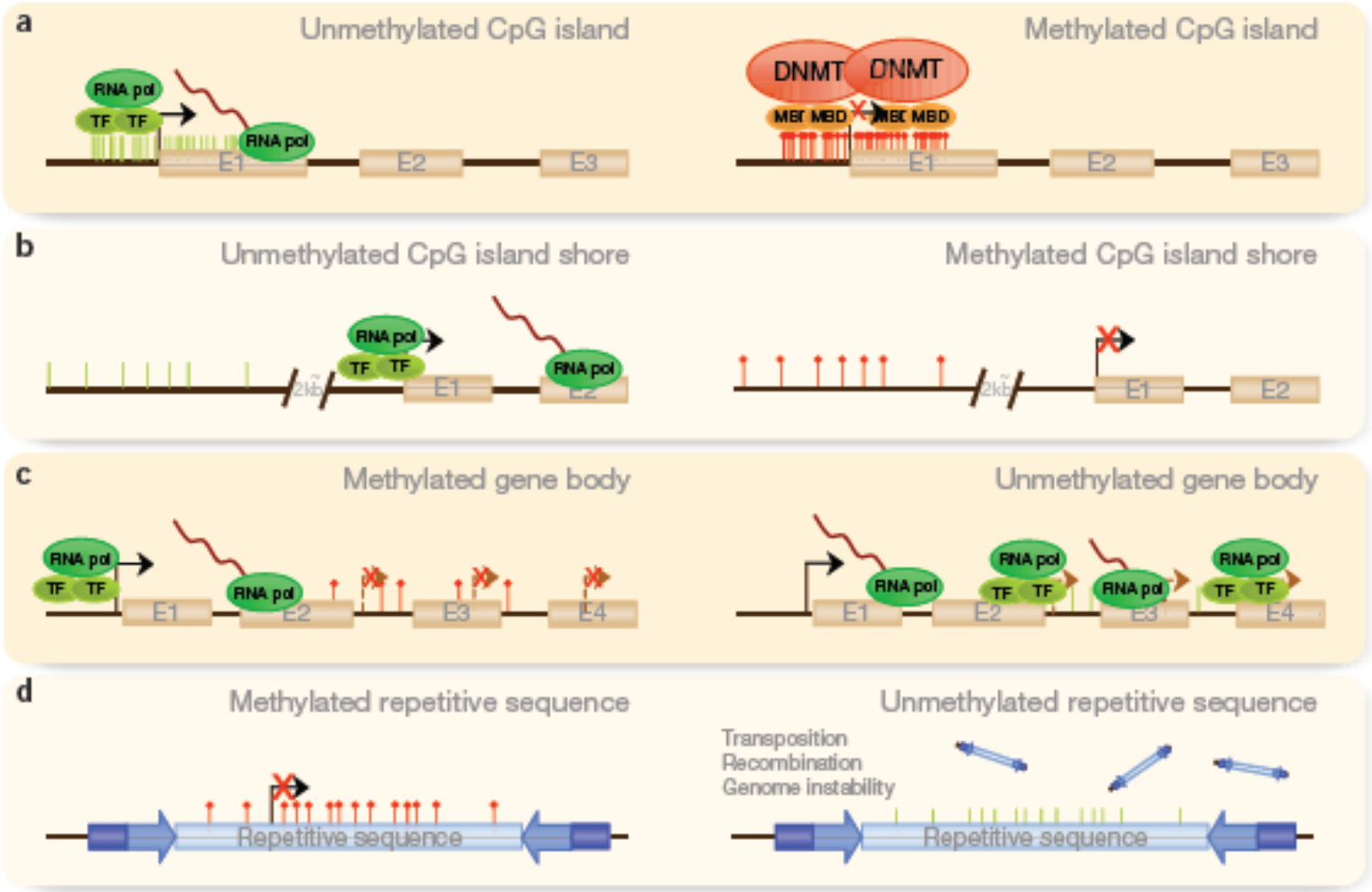
Functions of DNA methylation in mammals

1. Transcriptional gene silencing
2. Chromatin compaction
3. Genome stability
4. Suppression of homologous recombination between repeats
5. Genome defense
6. X chromosome inactivation (females)
7. Imprinting

Sites of Cytosine Methylation

1. **CpG islands:** regions of more than 200 bases (average of 1000 bases) with a G+C content of at least 50% (approximately 1% of the human genome, 60% of human gene promoters are associated with CpG islands.) CpG islands in promoters are usually unmethylated in normal cells. 6% become methylated in a tissue specific manner during early development or in differentiated tissues.
2. **CpG island shores:** regions of lower CpG density that lie in close proximity (2kb) of CpG islands. Most of the tissue –specific DNA methylation occurs in CpG island shores. 70% of the differentially methylated regions in reprogramming are associated with CpG island shores.
3. **Gene body:** seen in ubiquitously expressed genes, associated with transcriptional elongation.
4. **Repetitive elements:** protects chromosomal integrity by preventing reactivation of endoparasitic sequences that cause chromosomal instability, translocations, and gene disruption.

DNA Methylation and Gene Expression



STOP

STOP

Go

Figure 1 DNA methylation patterns. DNA methylation can occur in different regions of the genome. The alteration of these patterns leads to disease in the cells. The normal scenario is depicted in the left column and alterations of this pattern are shown on the right. (a) CpG islands at promoters of genes are normally unmethylated, allowing transcription. Aberrant hypermethylation leads to transcriptional inactivation. (b) The same pattern is observed when studying island shores, which are located up to 2 kb upstream of the CpG island. (c) However, when methylation occurs at the gene body, it facilitates transcription, preventing spurious transcription initiations. In disease, the gene body tends to demethylate, allowing transcription to be initiated at several incorrect sites. (d) Finally, repetitive sequences appear to be hypermethylated, preventing chromosomal instability, translocations and gene disruption through the reactivation of endoparasitic sequences. This pattern is also altered in disease.

DNA Methyltransferases (DNMTs)

DNMT family members:

DNMT1, DNMT2, DNMT3A, DNMT3B, DNMT3L

- DNMT3A and 3B: highly expressed in embryonic stem cells and thought to establish the pattern of methylation during embryonic development by catalyzing de novo methylation.

- DNMT1: prefers hemi-methylated DNA but can methylate DNA de novo. Is the most abundant DNMT and required for maintenance of methylation patterns. It is required to methylate hemi-methylated sites during replication (in conjunction with UHRF1 and PCNA).

Hydroxymethylation

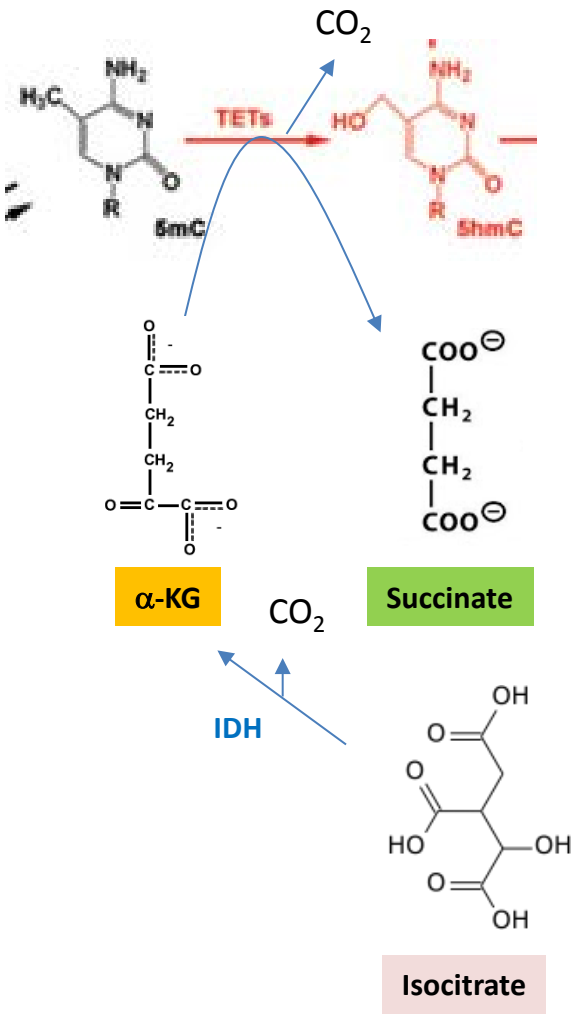
- TET proteins: family of hydroxylases
- Ten-eleven translocation 1 (TET1) is the founding member of this family
- Tet proteins are responsible for the conversion of 5mC to 5mhC in a 2-oxoglutarate- and Fe(II)-dependent manner

TET1

- Initially identified in acute myeloid leukemia (AML) as a fusion partner of the histone methyltransferase, mixed-lineage leukemia(MLL)
- Converts 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC)
- Can find 5mC oxidation derivatives on genomic DNA

IDHs (isocitrate dehydrogenases)

The cofactor α-ketoglutarate (α-KG) is absolutely required and plays a positive and critical role in the conversion of 5-mC to 5-hmC . Isocitrate dehydrogenases (IDHs) catalyze oxidative decarboxylation of isocitrate, producing α-ketoglutarate (α-KG) and CO_2



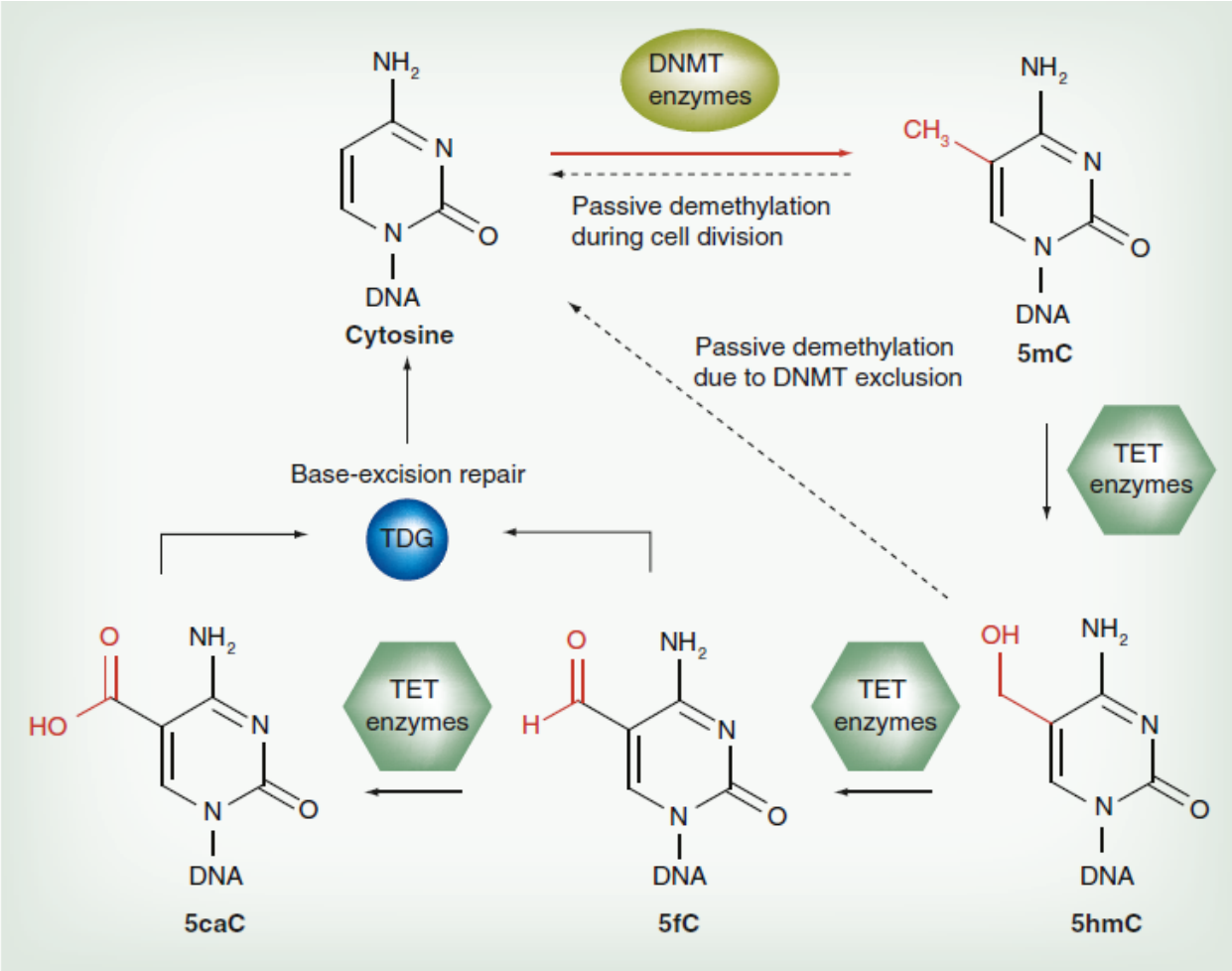
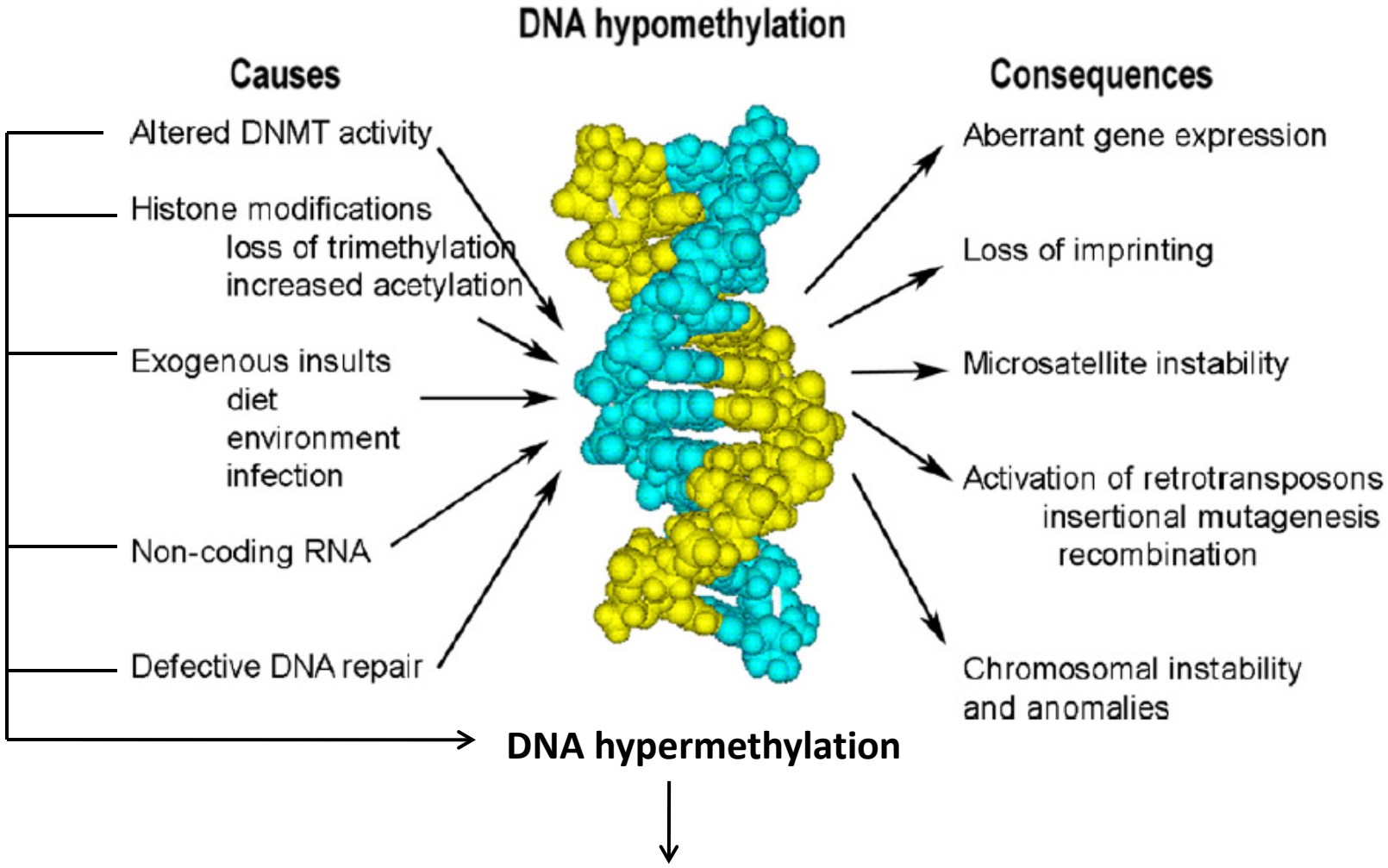


Figure 2. Proposed models of TET-mediated DNA demethylation pathways. Unmodified cytosine bases in a CpG dinucleotides can be directly methylated through the actions of the DNA methyltransferase enzymes. Demethylation is thought to be both passive (during cell division; dashed arrow) as well as active (through a series of enzymatic reactions). TET proteins can oxidize 5mC-modified bases into 5hmC and then onto 5fC and 5caC derivatives. The oxidized 5fC and 5caC bases are thought to provide suitable substrates for rapid demethylation to nonmodified cytosine via TDG-coupled base-excision repair. In contrast with these rapidly turned-over intermediates, 5hmC appears to remain stable in certain parts of the genome.
Black arrows: active methylation; dashed arrows: passive demethylation; red arrow: methylation.
5caC: 5-carboxylcytosine; 5fC: 5-formylcytosine; 5hmC: 5-hydroxymethylcytosine;
5mC: 5-methylcytosine.

Common Cancer Related Aberrations in DNA Methylation Patterns

- Massive global loss of DNA methylation(20% to 60% less overall 5-methylcytosine).
- Global hypomethylation occurs mainly at repetitive sequences, promoting chromosomal instability, translocations, gene disruption, and reactivation of endoparasitic sequences.
- Hypo-methylation at specific promoters can activate the aberrant expression of oncogenes and induce loss of imprinting (LOI)

- Hyper-methylation at the CpG islands of specific promoters can activate aberrant expression of oncogenes and induce loss of imprinting in some loci.
- Most hyper-methylation in cancer occurs in CpG island shores.
- Hyper-methylation patterns are tumor-type specific.



Silencing of tumor suppressors

Hypomethylation of LINEs in Cancer

- Hypomethylation of LINES has been observed in a number of cancers.
- In some cases, LINE demethylation is an early occurance (e.g. prostate and colon).
- For some cancers (leukemias, urothelial, ovarian,and breast) LINE demethylation increases with the degree of malignancy and correlates with clinical outcome.
- It is not clear whether LINE demethylation is a causative agent for cancer and what impact LINE hypomethylation has on clinical outcome.

Mechanisms that may lead to aberrant DNA methylation in cancer

1. **DNMT Expression and Activity**

- In animal models and in vitro, knockout and knockdown of DNMTs is associated with genomic hypomethylation and chromosomal abnormality and instability.
- Min mouse model (heterozygous for APC), knockout of DNMT1 leads to an overall decrease in the incidence of intestinal cancers but increase in hepatocellular carcinomas and adenomas.
- Levels of DNMT1 and DNMT3b are elevated in some cancers.

2. **Interaction with chromatin remodeling enzymes and other factors**

e.g. Gazin et al, Nature, 2007 identified factors induced by the oncogene, Ras which interact with DNMT1 to demethylate and silence the Fas pro-apoptotic gene,

3. **Involvement of RNA**:

RNA involved in region specific hypo-methylation of SpHK1 (sphingosine Kinase) gene (Imamura et al, BBRC, 1997).

- High affinity binding of an RNA to DNMT3A and 3B (Jeffrey et al, JBC, 2004)

  Garzon et al, Blood, 2009 found that in myloid leukemia, forced expression of mir-29b targets DNMT3A and 2B directly and DNMT1 indirectly. Mir-29b expression led to global DNA hypo-methylation and re-expression of tumor suppressor, p15.
-

3. **Defective Repair Mechanisms following exogenous insult-** Diet, UV radiation, chemicals may initiate DNA hypomethylation via DNA damage pathways. Underlying mechanisms remain speculative.

Table 3 | **Sensitive detection of cancer in plasma and serum using DNA methylation markers**

Disease	DNA source	Markers	Analytical sensitivity	Clinical sensitivity	Specificity	References
Bladder cancer	Plasma	*CDKN2A* (*ARF*)	13/15 (87%)	13/27 (48%)	N/A	64
	Plasma	*CDKN2A* (*INK4A*)	2/5 (40%)	2/27 (7%)	N/A	64
	Serum	*CDKN2A* (*INK4A*)	12/14 (86%)	19/86 (22%)	31/31 (100%)	151
Breast cancer	Plasma	*CDKN2A* (*INK4A*)	5/8 (63%)	5/35 (14%)*	N/A	152
	Plasma	*CDKN2A* (*INK4A*)	6/10 (60%)	6/43 (14%)*	N/A	61
Colorectal cancer	Serum	*MLH1*	3/9 (33%)	3/18 (17%)	N/A	63
	Serum	*CDKN2A* (*INK4A*)	14/20 (70%)	14/52 (27%)	44/44 (100%)‡	65
	Serum	*CDKN2A* (*INK4A*)	13/44 (30%)	13/94 (11%)	N/A	69
	Plasma	*CDKN2A* (*INK4A*)	21/31 (68%)	21/58 (36%)	N/A	73
Oesophageal cancer	Plasma (AC)	*APC*	13/48 (27%)	13/52 (25%)	54/54 (100%)‡	52
	Plasma (SCC)	*APC*	2/16 (13%)	2/32 (6%)	54/54 (100%)‡	52
	Serum (SCC)	*CDKN2A* (*INK4A*)	7/31 (23%)	7/38 (18%)	N/A	153
Gastric cancer	Serum	*CDH1*	31/41 (76%)	31/54 (57%)	30/30 (100%)	70
	Serum	*CDKN2A* (*INK4A*)	28/36 (78%)	28/54 (52%)	30/30 (100%)	70
	Serum	*CDKN2B* (*INK4B*)	30/37 (81%)	30/54 (56%)	30/30 (100%)	70
	Serum	*DAPK1*	26/38 (68%)	26/54 (48%)	30/30 (100%)	70
	Serum	*GSTP1*	8/10 (80%)	8/54 (15%)	30/30 (100%)	70
	Serum	Panel of five	45/54 (83%)	45/54 (83%)	30/30 (100%)	70
Head and neck cancer	Serum	*CDKN2A* (*INK4A*)	8/26 (31%)	8/95 (8%)	N/A	68
	Serum	*DAPK1*	3/17 (18%)	3/95 (3%)	N/A	68
	Serum	*MGMT*	14/31 (45%)	14/95 (15%)	N/A	68
	Serum	Panel of three	21/52 (40%)	21/95 (22%)	N/A	68
	Plasma (nasopharyngeal)	*DAPK1*	6/12 (50%)	N/A	N/A	71
Liver cancer	Plasma/serum	*CDKN2A* (*INK4A*)	13/16 (81%)	13/22 (45%)	48/48 (100%)	66
	Plasma/serum	*CDKN2B* (*INK4B*)	4/16 (25%)	4/25 (16%)	35/35 (100%)	154
	Plasma/serum	Panel of two	17/23 (74%)	17/25 (68%)	35/35 (100%)	154
Lung cancer	Serum (NSCLC)	*CDKN2A* (*INK4A*)	3/9 (33%)	3/22 (14%)	N/A	67
	Serum (NSCLC)	*DAPK1*	4/5 (80%)	4/22 (18%)	N/A	67
	Serum (NSCLC)	*GSTP1*	1/2 (50%)	1/22 (5%)	N/A	67
	Serum (NSCLC)	*MGMT*	4/6 (67%)	4/22 (18%)	N/A	67
	Serum (NSCLC)	Panel of four	11/15 (73%)	11/22 (50%)	N/A	67
	Plasma	*CDKN2A* (*INK4A*)	1/10 (10%)	N/A	N/A	155
	Plasma/serum	*APC*	N/A	42/89 (47%)	50/50 (100%)	89
	Plasma	*CDKN2A* (*INK4A*)	64/73 (88%)	77/105 (73%)§	N/A	72
	Plasma (NSCLC)	*CDKN2A* (*INK4A*)	12/22 (55%)	12/35 (34%)	15/15 (100%)	156
Prostate cancer	Plasma/serum	*GSTP1*	12/16 (75%)	23/33 (70%)	22/22 (100%)‡	62
	Plasma	*GSTP1*	25/63 (40%)	25/69 (36%)	31/31 (100%) ‡	76

DNA Methylation and Patient Survival in Lung Cancer

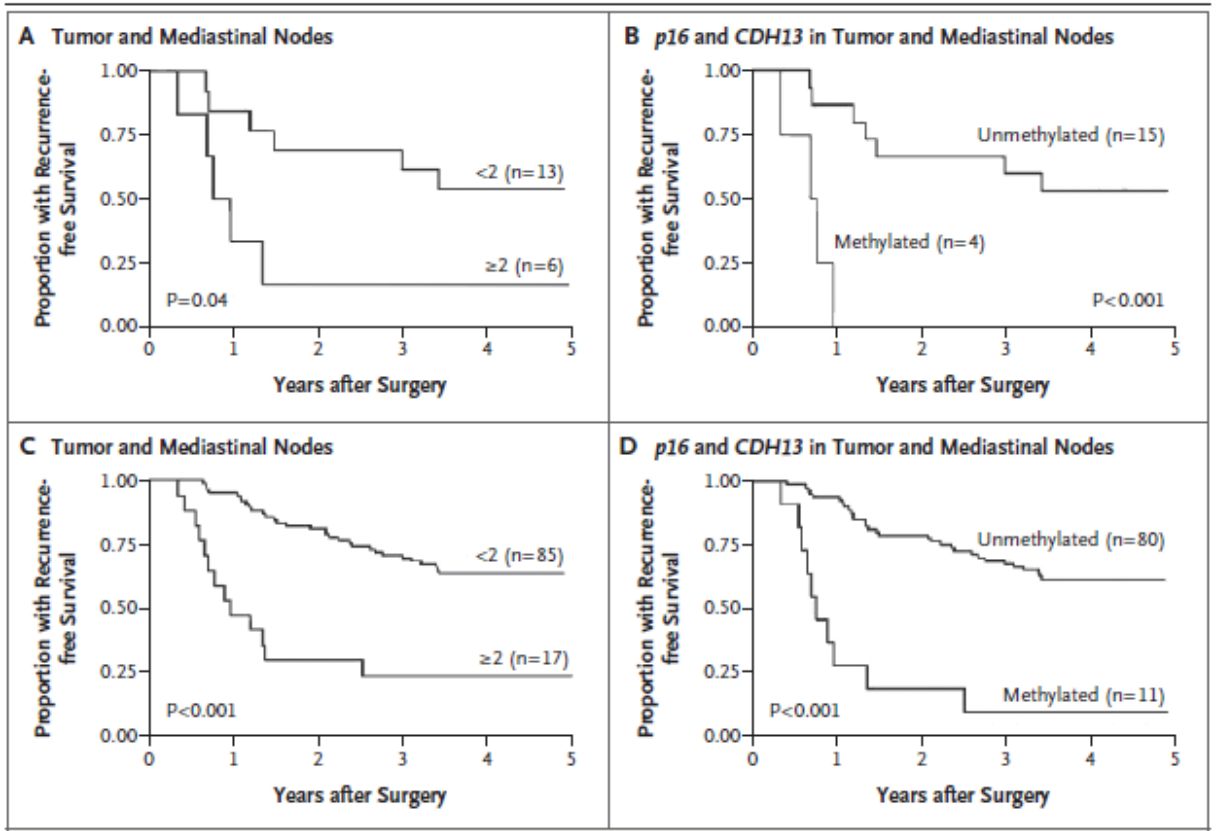


Figure 3. Kaplan–Meier Estimates of Recurrence-free Survival in the Validation Cohort or the Validation and Original Cohorts, According to the Site and Number or Presence or Absence of Methylated Genes.

Data are reported for a four-gene panel consisting of the cyclin-dependent kinase inhibitor 2A gene *p16*, the H-cadherin gene *CDH13*, the *Ras* association domain family 1 gene *RASSF1A*, and the adenomatous polyposis coli gene *APC*. Panels A and B show data for the independent validation cohort of 20 case patients and controls. Panels C and D show data for the combined original and validation cohorts (total, 187 patients). The numbers of patients vary among panels because not every sample was informative for every gene.

Brock et al, New England Journal of Medicine, 2008

Box 1. Summary of changes in 5-hydoxymethylcytosine patterns and possible underlying mechanisms seen in cancer.

- Cancer-associated hydroxymethylcytosine patterns
- General loss of 5hmC
- Cancer-specific redistribution with enrichment, particularly at oncogenic gene activators
- Possible mechanisms for 5hmC changes in cancer
- Replication-related passive demethylation and loss of hydroxymethylcytosine
- Misexpression or mutation of TET enzymes, which are responsible for the oxidative conversion of 5mC to 5hmC
- Inhibition of the essential TET cofactor, α-ketoglutarate, through mutation of isocitrate dehydrogenase or other Krebs cycle enzymes, such as fumerate hydratase or succinate dehydrogenase

5mC: 5-methylcytosine; 5hmC: 5-hydroxymethylcytosine.

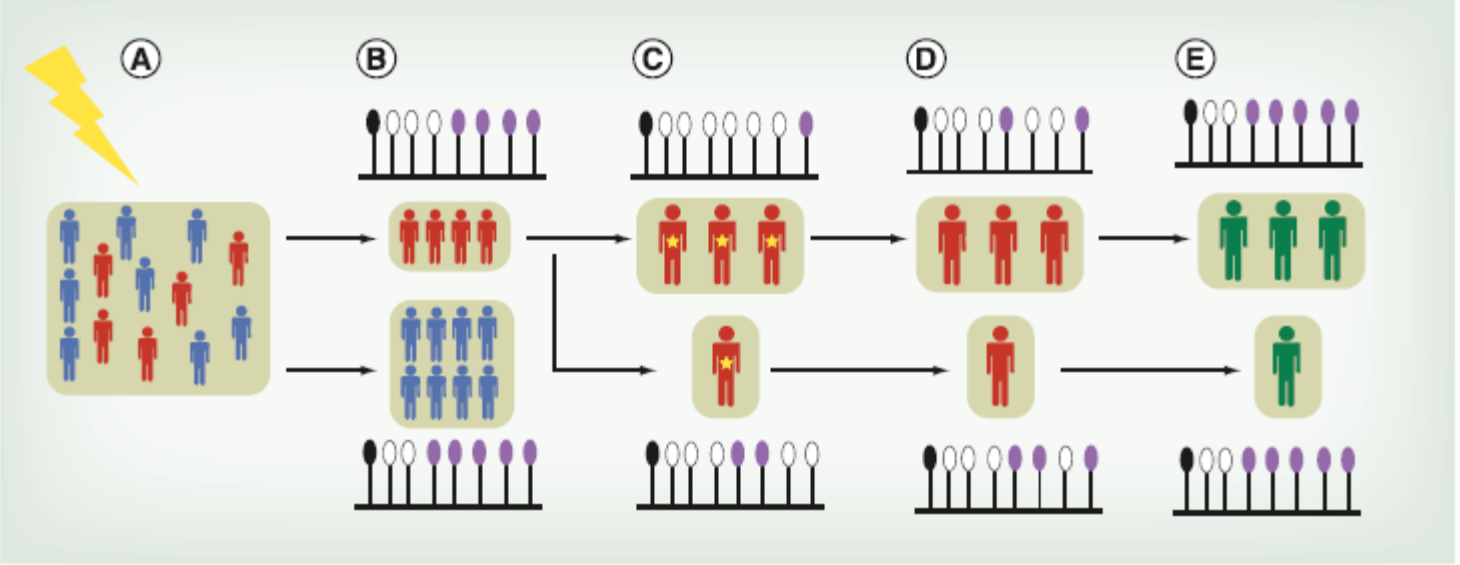


Figure 4. Potential model of 5-hydroxymethylcytosine biomarker use in the diagnosis and management of cancer. (A) Cohort exposed to risk factor for disease. **(B)** Red population have 5-hydroxymethylcytosine (5hmC) signature suggestive of sensitivity to this risk factor (black lollipops: 5-methylcytosine-marked CpGs; purple lollipops: 5hmC marked; white lollipops: unmarked CpGs). These patients can undergo close surveillance to allow early detection or pre-emptive treatment, if available. Blue patients can be reassured of low risk. **(C)** Patients develop cancer and can be stratified into treatment-sensitive or treatment-resistant groups to allow personalized therapy. **(D)** Response to treatment can be followed. **(E)** Return to 'normal' 5hmC tissue signature on cure.

Methods for detecting DNA methylation

- Methylation sensitive restriction enzymes
- Immunoprecipitation based enrichment assays
- Methylation sensitive PCR (MSP)
- Bisulfite conversion

Methylation-Sensitive Restriction Digestion Assay

- Cost effective method for initial screening

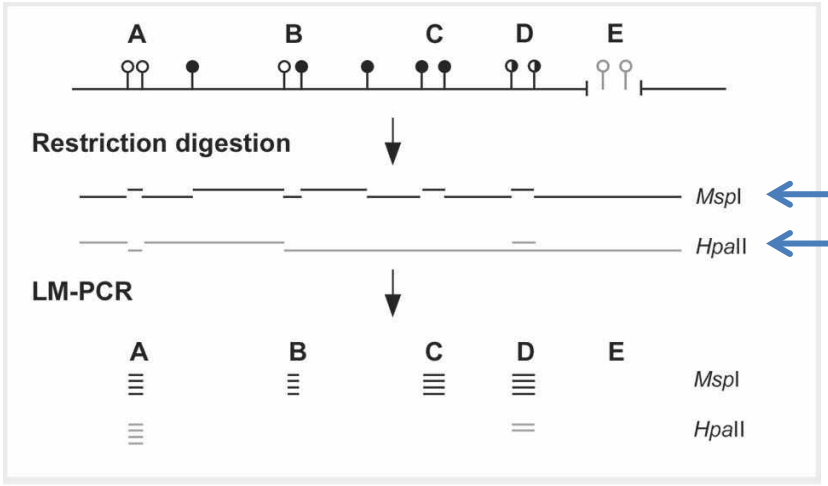
HELP Assay

Use restriction enzymes (HpaII and MspI) to determine methylation status

- HpaII Tiny Fragments (HTFs)
- HTF enrichment by ligation-mediated PCR
- Combine with microarrays

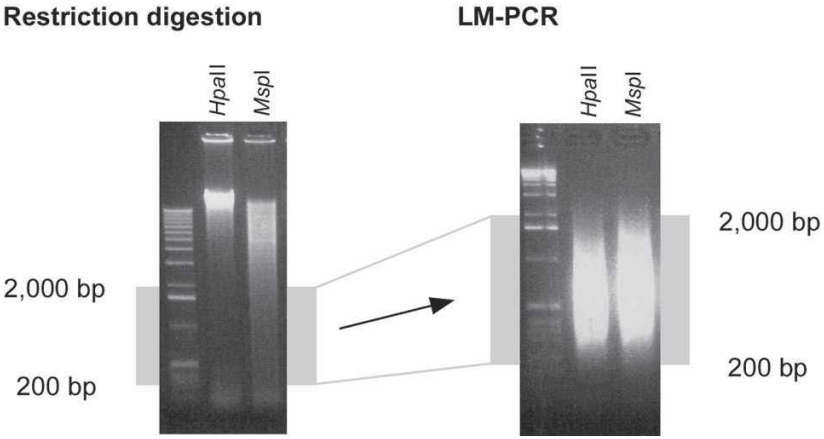
HELP Assay

(5-CCGG-3) (< than 12% of these sites are in CpG islands



Methylation insensitive

Methylation sensitive



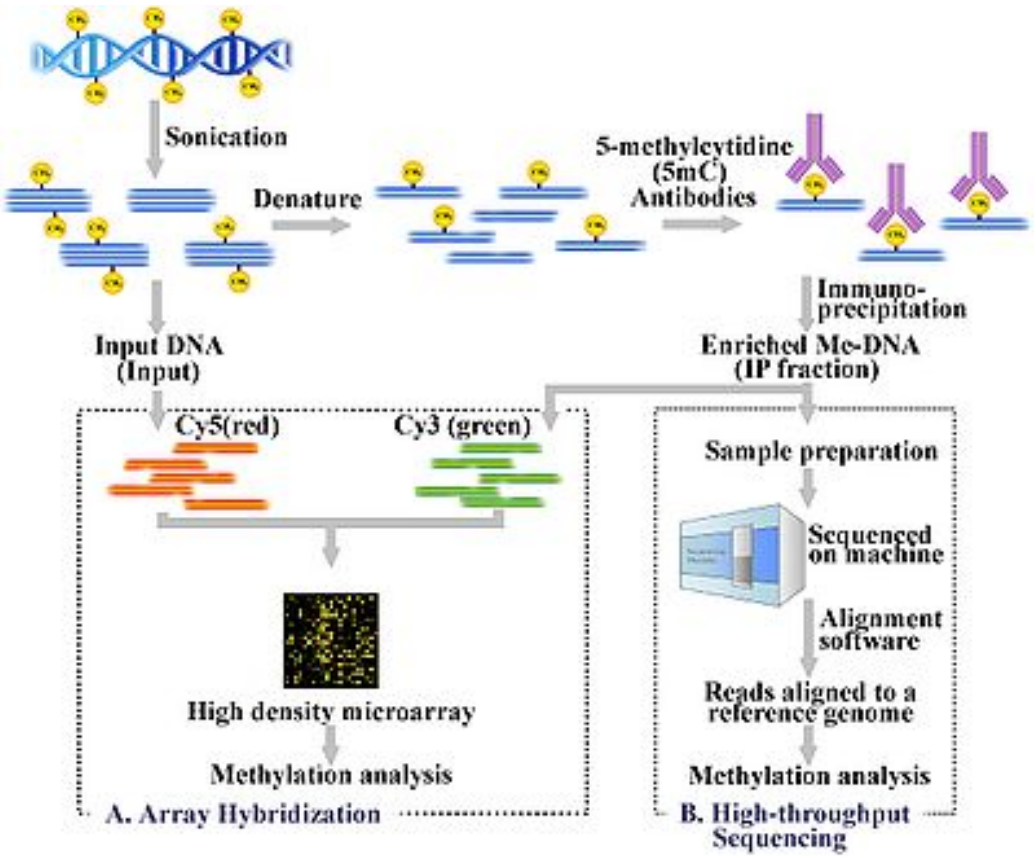
Generate HpaII/MspI

Khulan et al Genome Research, 2012

Immunoprecipitation Based Enrichment Assays

MeDip

- Antibodies directed against methylated CpGs are used to enrich DNA in methylated sequences relative to control DNA.
- The resulting intensity ratio represents a ratio of methylated fragments over the total control and positive values are interpreted as enrichment for methylation.
- Although this method is not constrained to measuring methylation in recognition sites, the drawback is a lack of specificity in low CpG dense regions due to noise.



Bisulfite Conversion

- Bisulfite (HSO_3^-): converts unmethylated cytosines into uracil
- Uracil is converted to thymine following PCR amplification while leaving methylated cytosines unconverted
- Bisulfite conversion offers single CpG resolution when PCR product is analyzed by PCR, microarray, or sequencing.

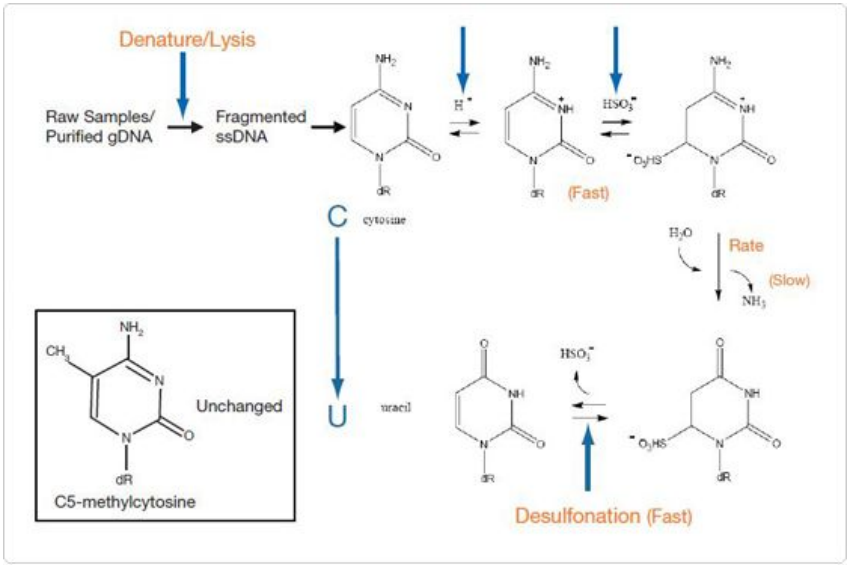
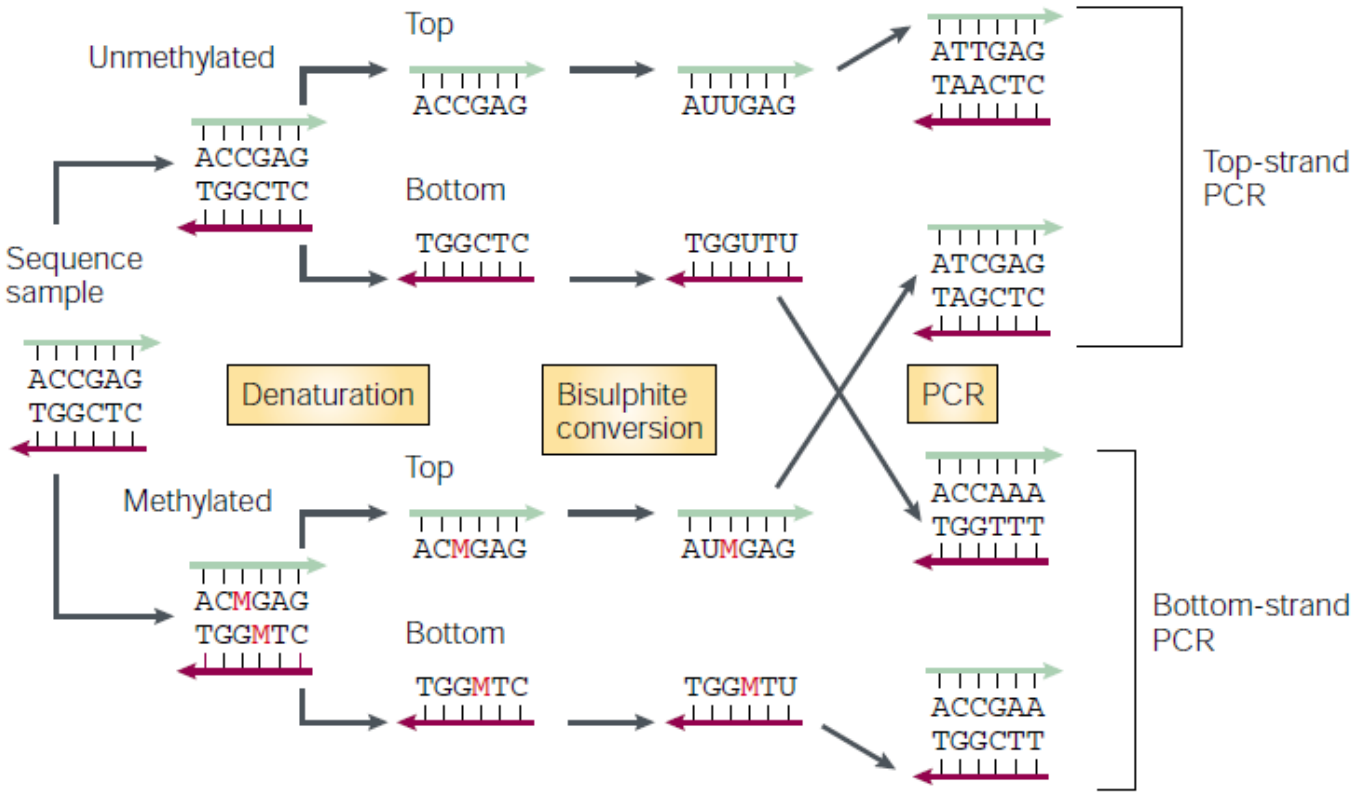
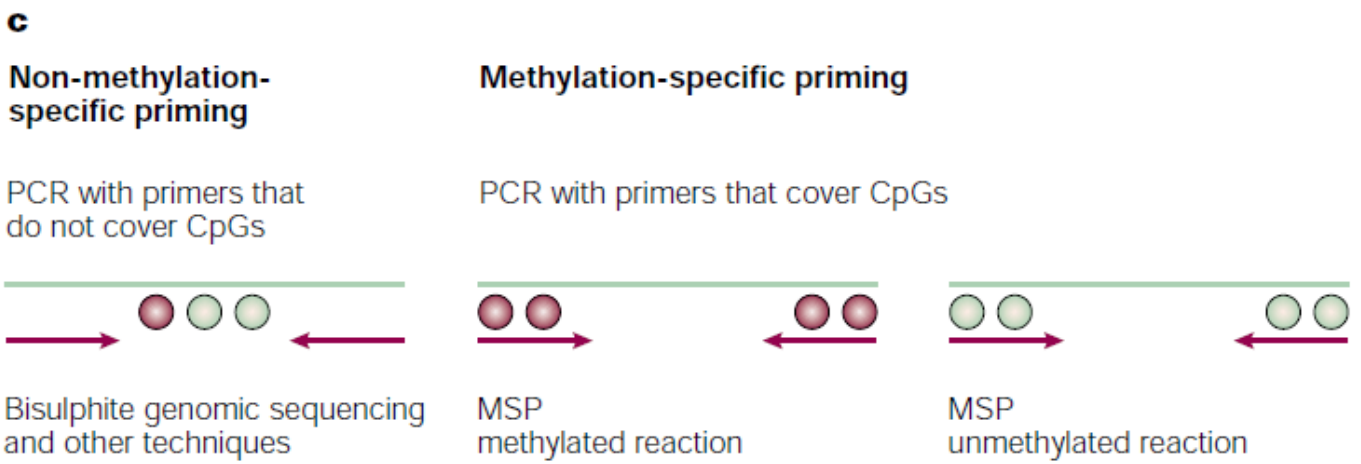


Figure 1. Cytosine Conversion Chemistry .

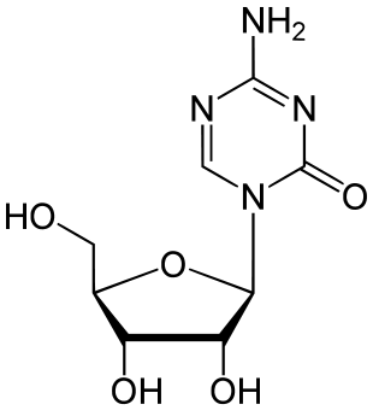




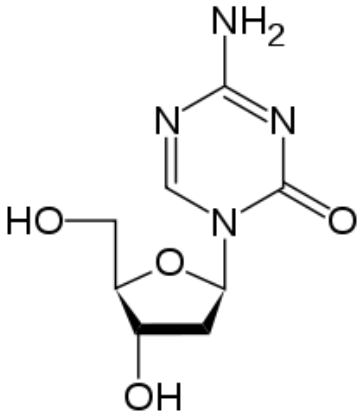
Laird, Nature Rev. Cancer, 2003

Epigenetic Treatment

- DNA Methylation Inhibitors: nucleoside analogues that exert their demethylating activity through the establishment of an irreversible covalent bond with DNMTs after their incorporation into DNA.



5-Azacytidine



5-Aza-2’deoxy-cytidine

Mechanisms of Action

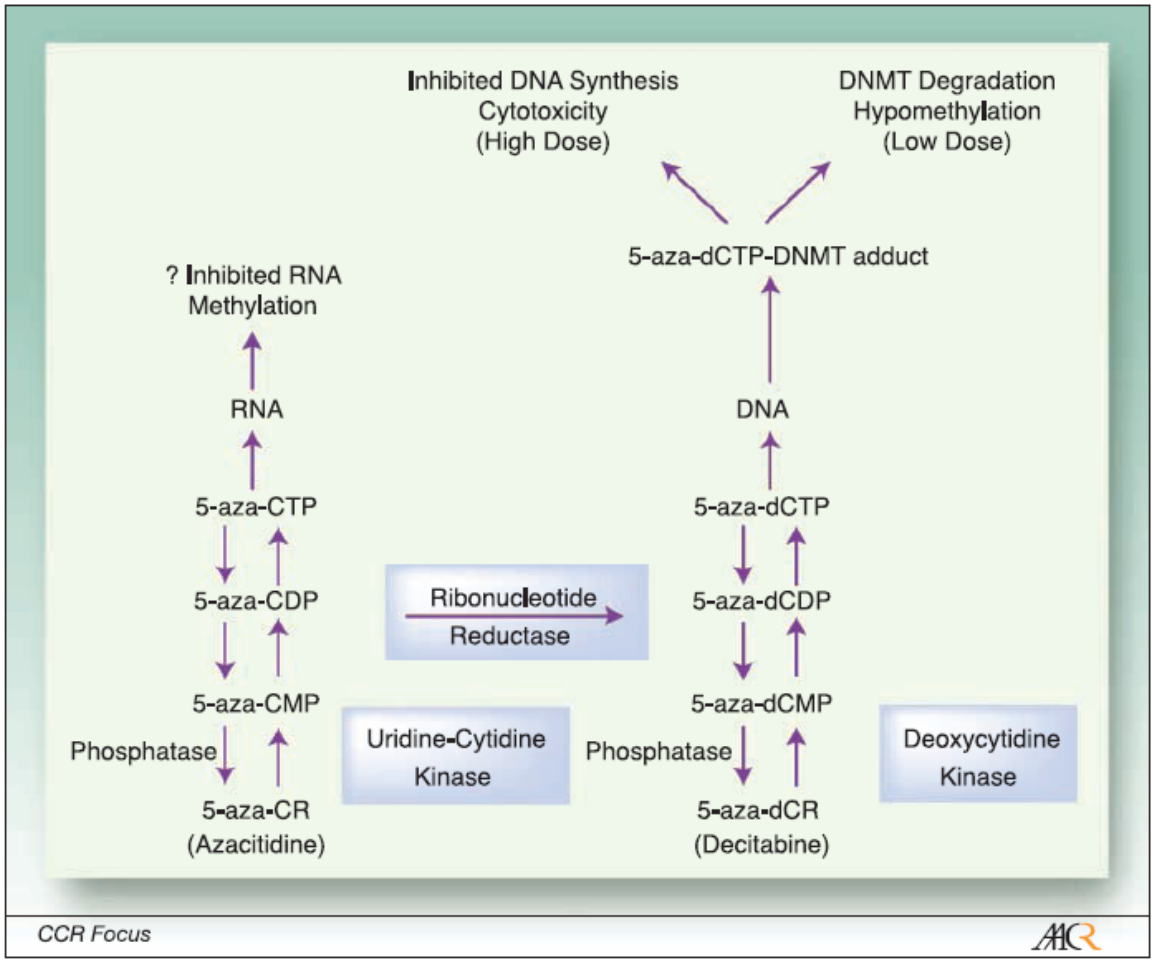


Fig. 2. Mechanisms of action of hypomethylating nucleoside analogs. Azacitidine and decitabine are efficiently incorporated into cells by specialized transporters, following which their metabolism diverge. They are phosphorylated by different enzymes, eventually to 5-aza-CTP, which incorporates into RNA and has poorly defined effects there and 5-aza-dCTP, which incorporates into DNA. A fraction of 5-aza-CDP is also converted to 5-aza-dCDP. Once incorporated into DNA, 5-aza-dCTP forms irreversible covalent bonds with DNMTs, which result in bulky DNA-protein adducts and inhibition of DNA synthesis. At high doses, this results in cell death (and is therefore a cytotoxic intervention). At lower doses, the complexes are excised and degraded by the proteosome. DNA is repaired, following which DNA synthesis resumes in the absence of DNMTs, resulting in hypomethylation of newly synthesized DNA.

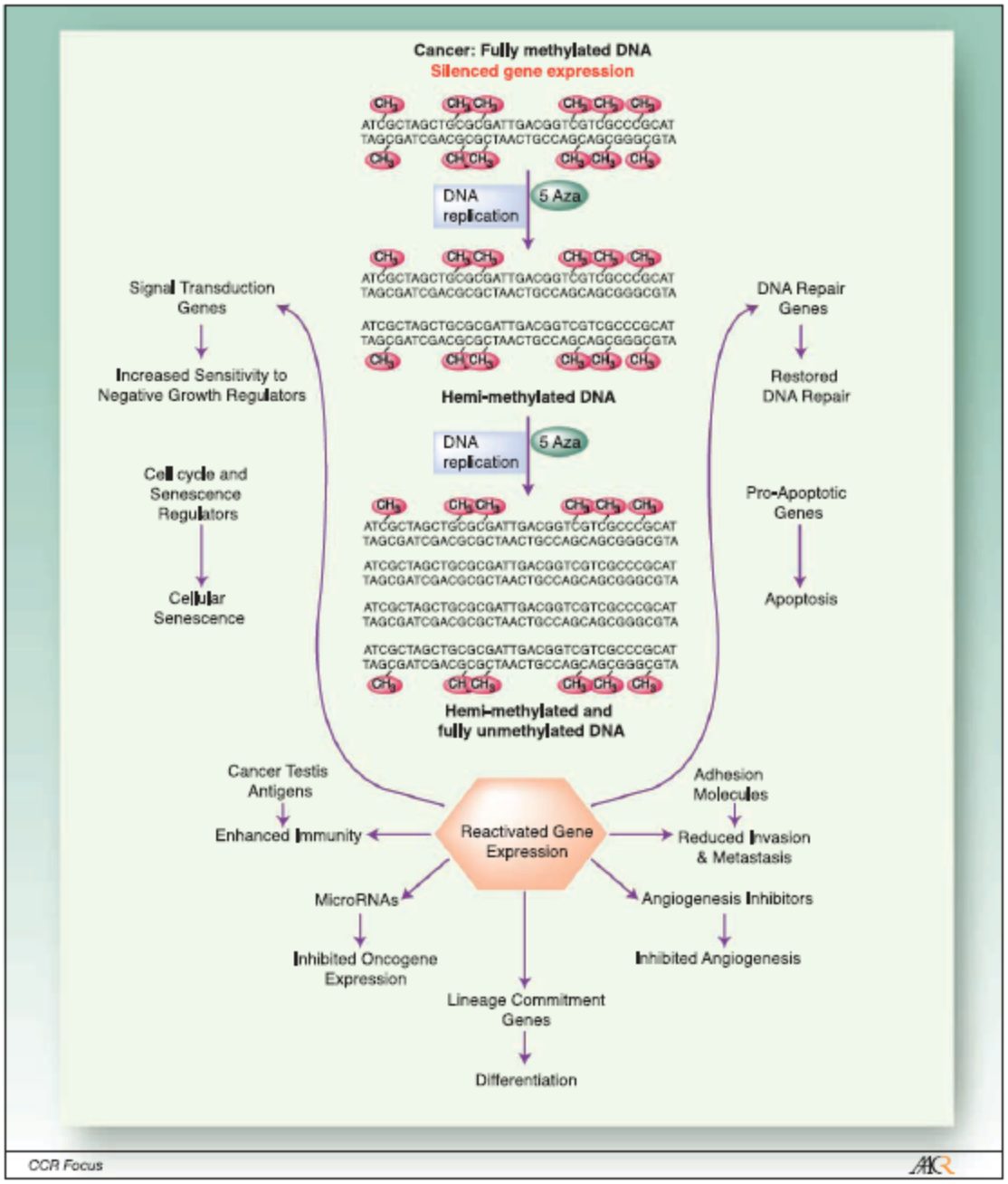
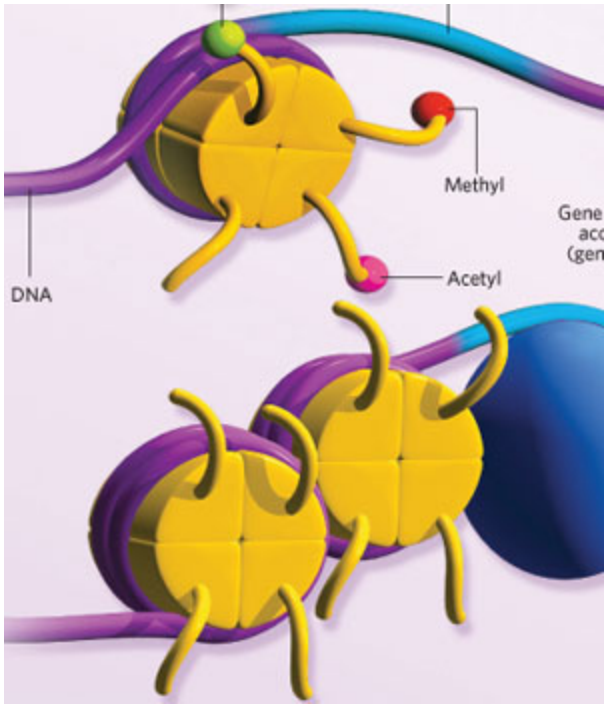


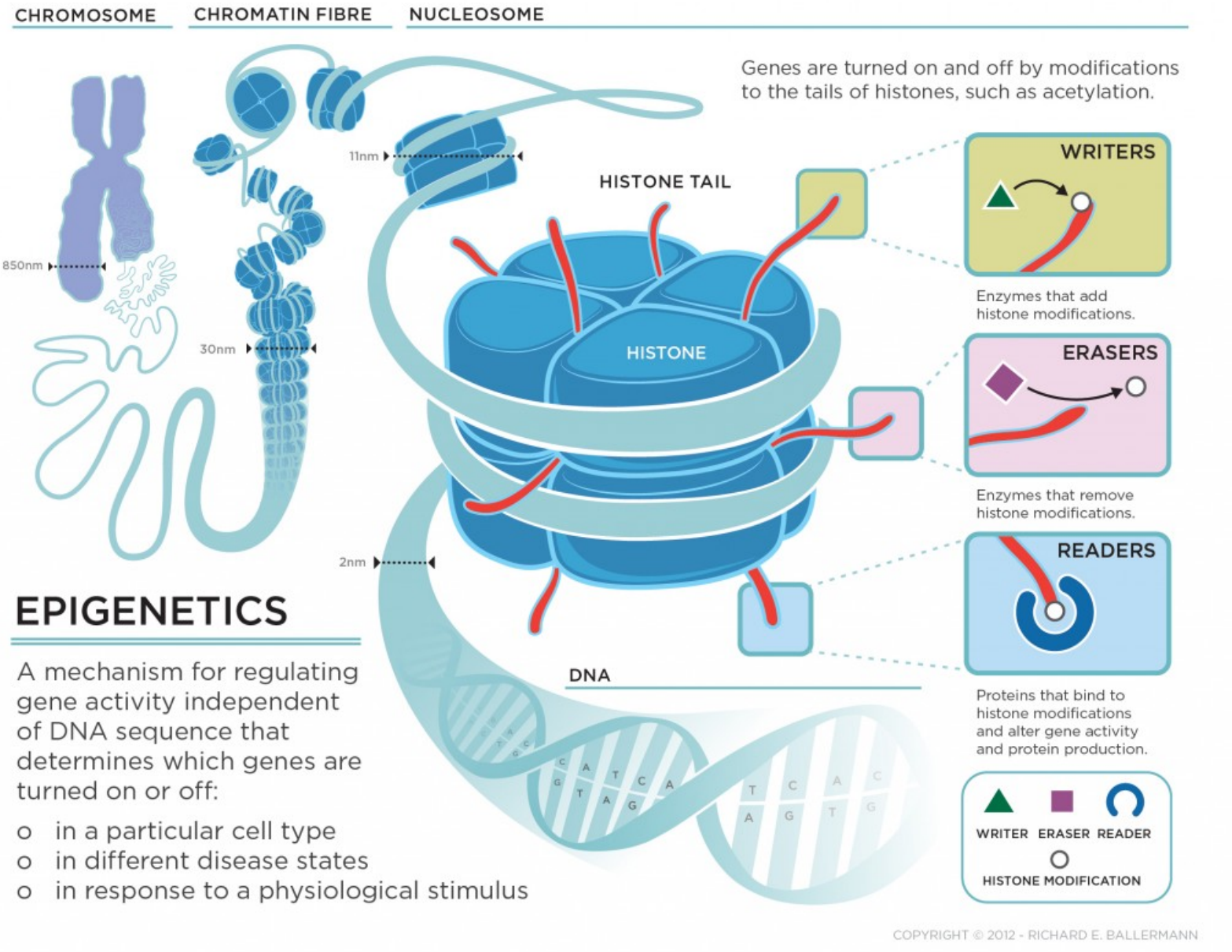
Fig. 3. Pleiotropic therapeutic effects of DNA methylation inhibition and gene reactivation in cancer. DNA methylation is maintained postreplication by the action of DNA methyltransferases. DAC and AZA lead to degradation of the main DNA methyltransferases, and continued replication results in passive demethylation that eventually results in reactivated gene expression. Activated gene expression, in turn, has effects on multiple different pathways, each of which could contribute to a clinical response.

Chromatin as an Epigenetic Regulator

1. Histone Modifications

2. Nucleosome Positioning





…and ATP dependent chromatin remodeling enzymes

Histone Modifications

Figure 3 Histone modifications. All histones are subject to post-transcriptional modifications, which mainly occur in histone tails. The main post-transcriptional modifications are depicted in this figure: acetylation (blue), methylation (red), phosphorylation (yellow) and ubiquitination (green). The number in gray under each amino acid represents its position in the sequence.

Acetylation and Methylation of Histone N terminal tails

Nomenclature:

Histone-Position-modification-number of modifications

H(1,2,3,4)-K/R-ac/Me-_2,3

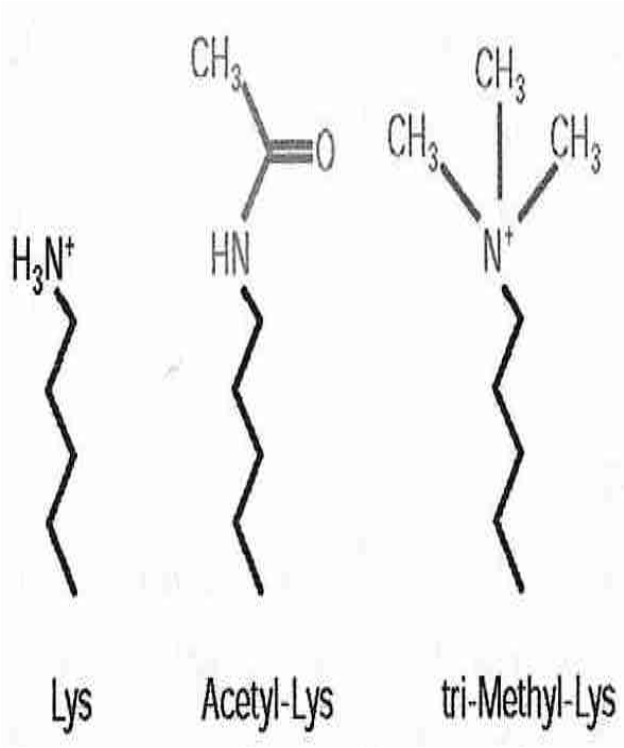
e.g. H3K4

H3K4me

H3K4me2

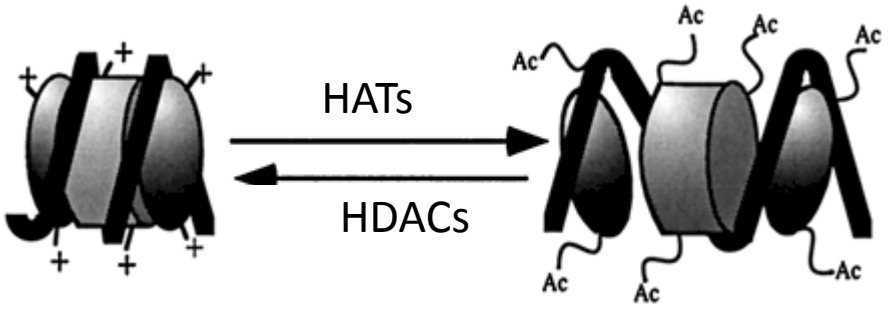
H3K4me3

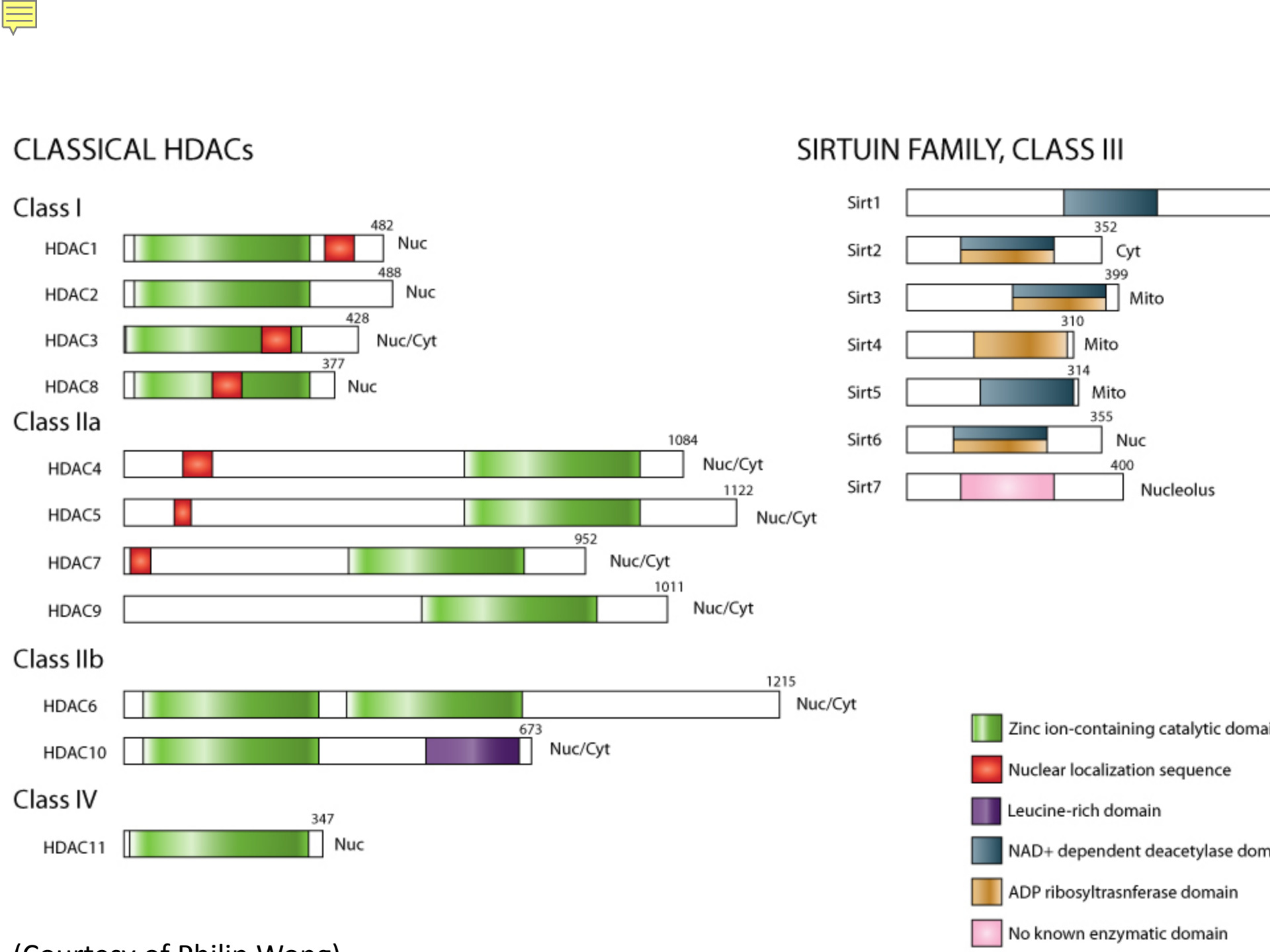
H3K9ac



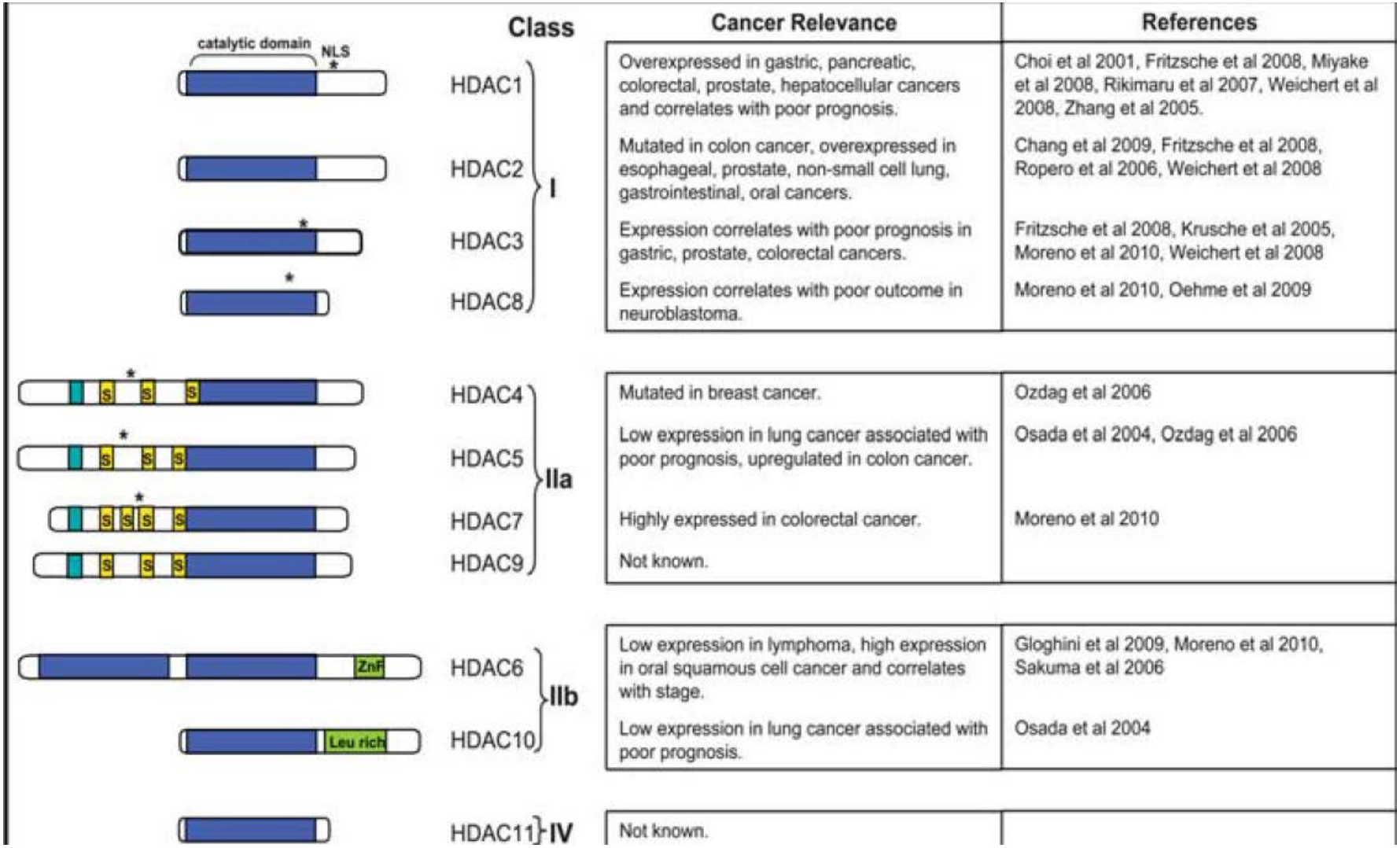
Histone Modifying Enzymes

- Histone Acetyl Transferases (HATs)/Histone Deacetylases (HDACs)





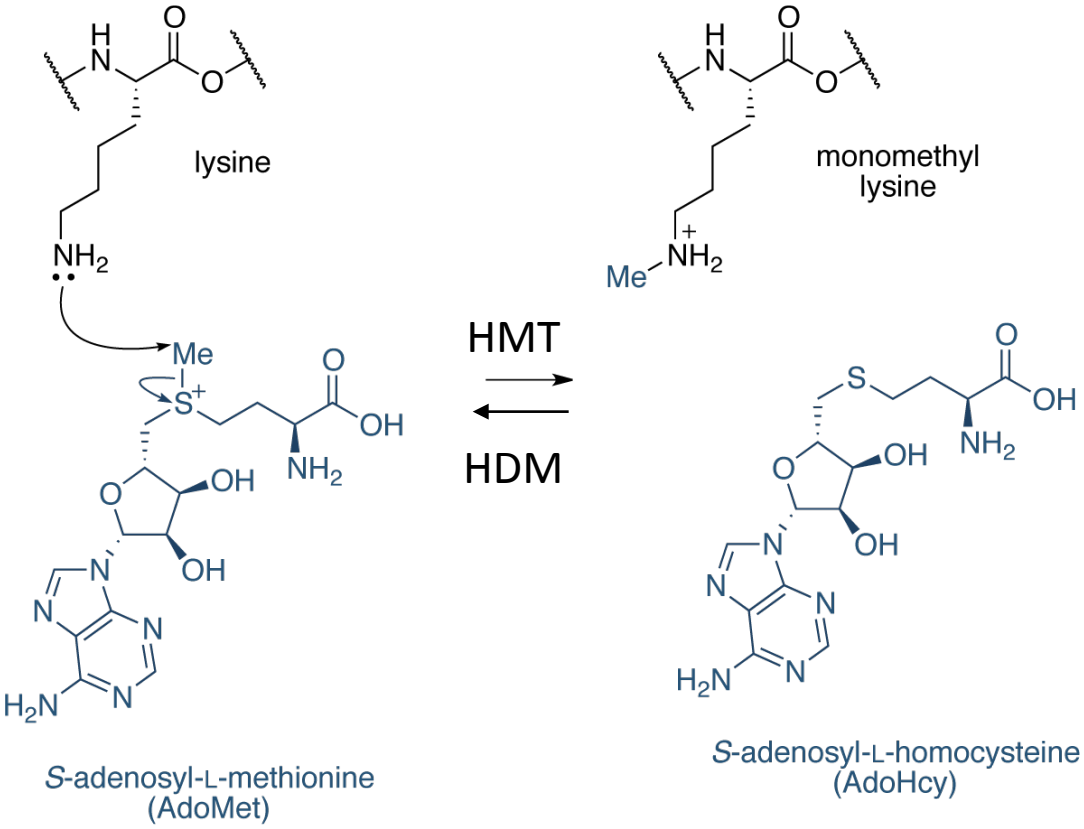
(Courtesy of Philip Wang)

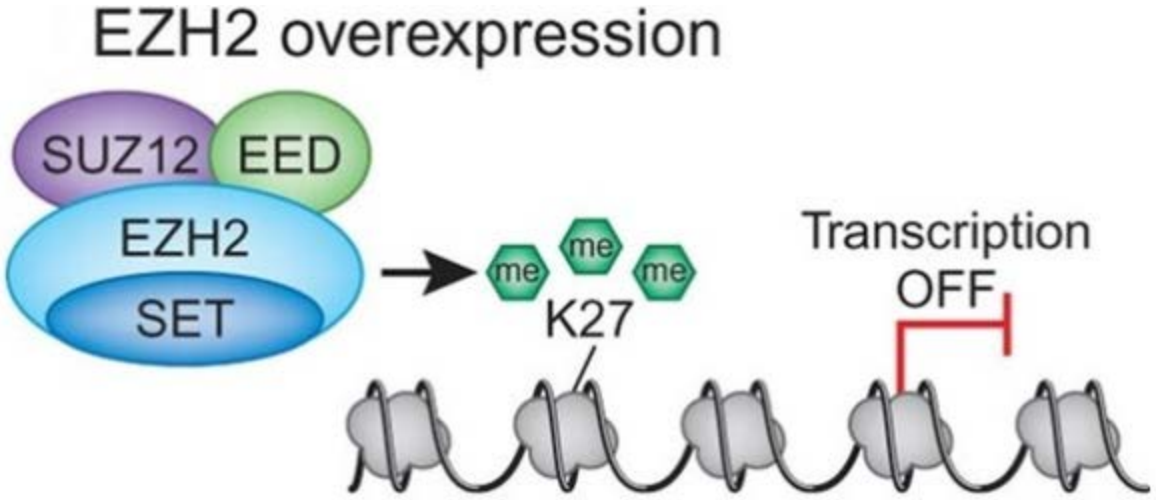


Class	Cancer Relevance	References
HDAC1	Overexpressed in gastric, pancreatic, colorectal, prostate, hepatocellular cancers and correlates with poor prognosis.	Choi et al 2001, Fritzsche et al 2008, Miyake et al 2008, Rikimaru et al 2007, Weichert et al 2008, Zhang et al 2005.
HDAC2 (I)	Mutated in colon cancer, overexpressed in esophageal, prostate, non-small cell lung, gastrointestinal, oral cancers.	Chang et al 2009, Fritzsche et al 2008, Ropero et al 2006, Weichert et al 2008
HDAC3	Expression correlates with poor prognosis in gastric, prostate, colorectal cancers.	Fritzsche et al 2008, Krusche et al 2005, Moreno et al 2010, Weichert et al 2008
HDAC8	Expression correlates with poor outcome in neuroblastoma.	Moreno et al 2010, Oehme et al 2009
HDAC4	Mutated in breast cancer.	Ozdag et al 2006
HDAC5 (IIa)	Low expression in lung cancer associated with poor prognosis, upregulated in colon cancer.	Osada et al 2004, Ozdag et al 2006
HDAC7	Highly expressed in colorectal cancer.	Moreno et al 2010
HDAC9	Not known.	
HDAC6	Low expression in lymphoma, high expression in oral squamous cell cancer and correlates with stage.	Gloghini et al 2009, Moreno et al 2010, Sakuma et al 2006
HDAC10 (IIb)	Low expression in lung cancer associated with poor prognosis.	Osada et al 2004
HDAC11 (IV)	Not known.	

Histone Modifying Enzymes

- Histone Methyltransferases (HMTs)/Histone Demethylases (HDMs)




EZH2 overexpression
SUZ12
EED
EZH2
SET
me
me
me
K27
Transcription
OFF

EZH2 mediated gene silencing in Cancer

Table 1. List of repressed targets downstream of EZH2 in cancer

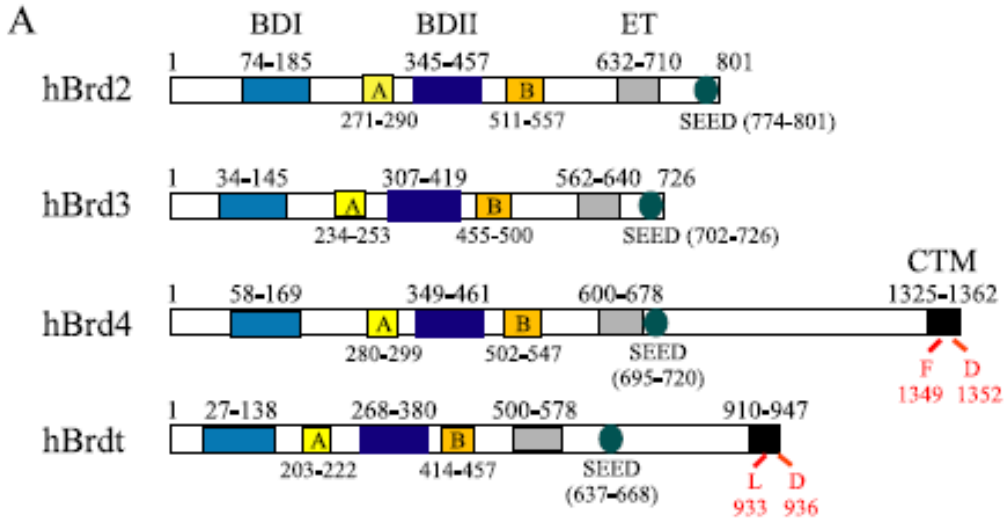
EZH2 targets in prostate cancer			
Target	Function	Contribution to carcinogenesis	Reference
DAB2IP	Inhibition of NF-κB/Ras pathway	Transformation, proliferation and invasion	Chen et al., 2005; Min et al., 2010
ADRB2	B-adrenergic signaling	Transformation and invasion	Yu et al., 2007
CDH1	Cell-cell adhesion	Invasion	Cao et al., 2008
PSP94	Inhibits MMP secretion	Invasion	Beke et al., 2007
SLIT2	Chemorepellent protein	Proliferation and invasion	Yu et al., 2010
TIMP2/3	ECM degradation	Invasion	Shin and Kim, 2012
RKIP	Inhibition of Raf and NF-κB pathways	Invasion	Ren et al., 2012
PCAT-1	Transcriptional repressor lincRNA	Proliferation	Prensner et al., 2011
EZH2 targets in other cancer types			
Target	Function	Cancer type	Reference
FOXC1	Transcription factor for differentiation	Breast cancer	Du et al., 2012
RAD51	DNA damage repair protein	Breast cancer	Chang et al., 2011
BMPR1B	Astroglial differentiation	Glioblastoma	Lee et al., 2008
VASH1	Inhibition of angiogenesis	Ovarian cancer	Lu et al., 2010
DKK1	Wnt signaling antagonist	Lung cancer	Hussain et al., 2009

BET-family of Bromodomain containing proteins

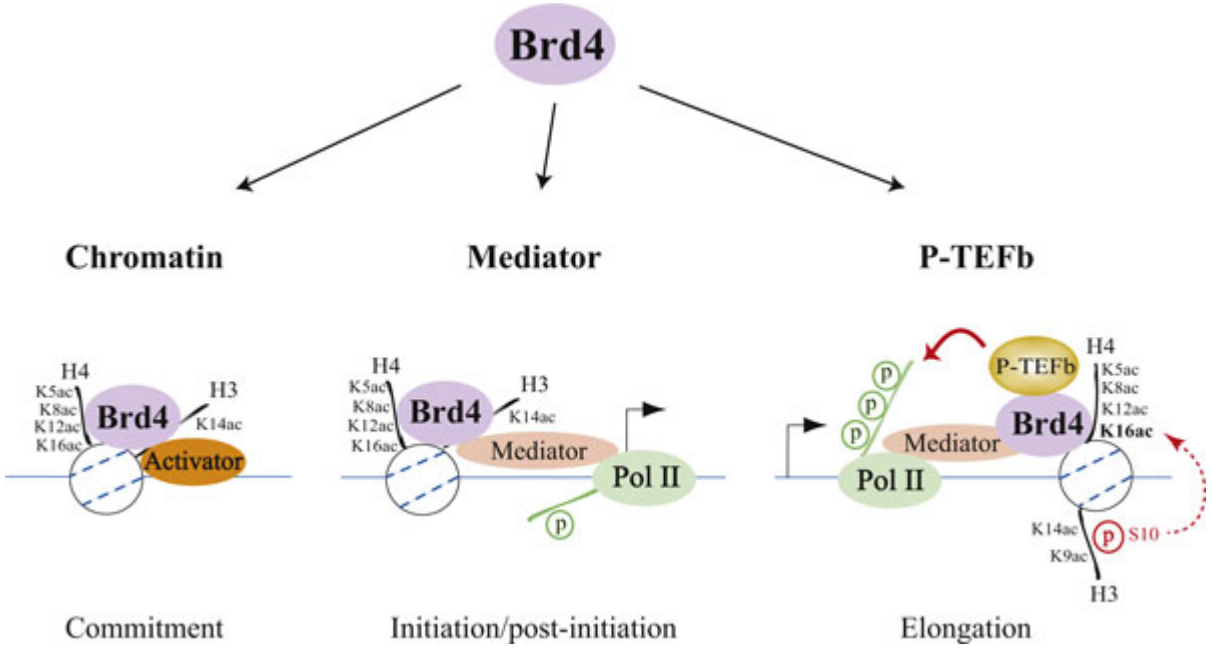
Bromodomains (BRDs) are epigenetic readers that recognize acetylated-lysine (KAc) on proteins and are implicated in a number of diseases.

<u>The BET (bromodomain and external domain family:</u>

BRD2
BRD3
BRD4
BRDT



BRD4 Promotes Transcription



Nucleosome Positioning

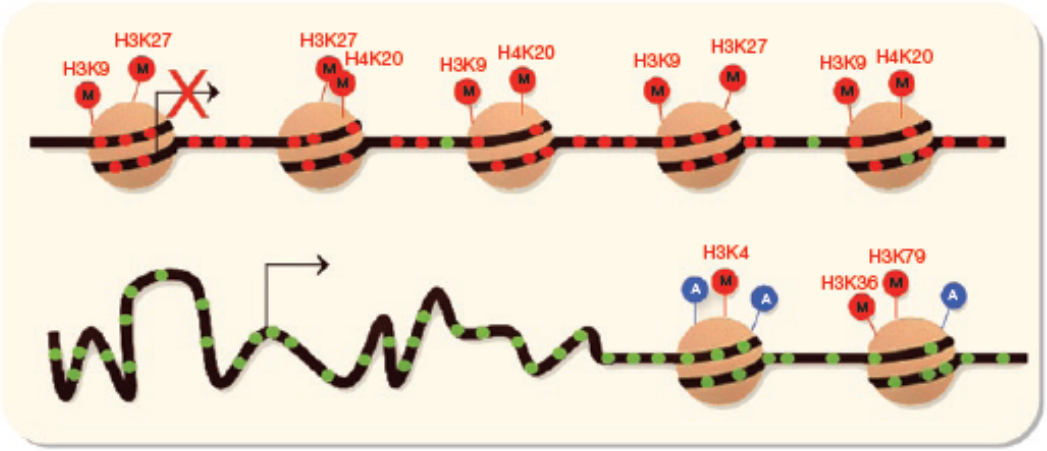


Figure 4 Nucleosome positioning patterns. Nucleosome positioning plays an important role in transcriptional regulation. Transcriptionally active gene promoters possess a nucleosome-free region at the 5′ and 3′ untranslated region, providing space for the assembly and disassembly of the transcription machinery. The loss of a nucleosome directly upstream of the TSS is also necessary for gene activation, whereas the occlusion of this position leads to transcription repression. DNA methylation regulates transcription, and thus interferes with nucleosome positioning. Methylated DNA seems to be associated with 'closed' chromatin domains, where DNA is condensed into strictly positioned nucleosomes, thereby impeding transcription. Conversely, unmethylated DNA is associated with 'opened' chromatin domains, which allow transcription.

Enzymes that Regulate Nucleosome Positioning/structure

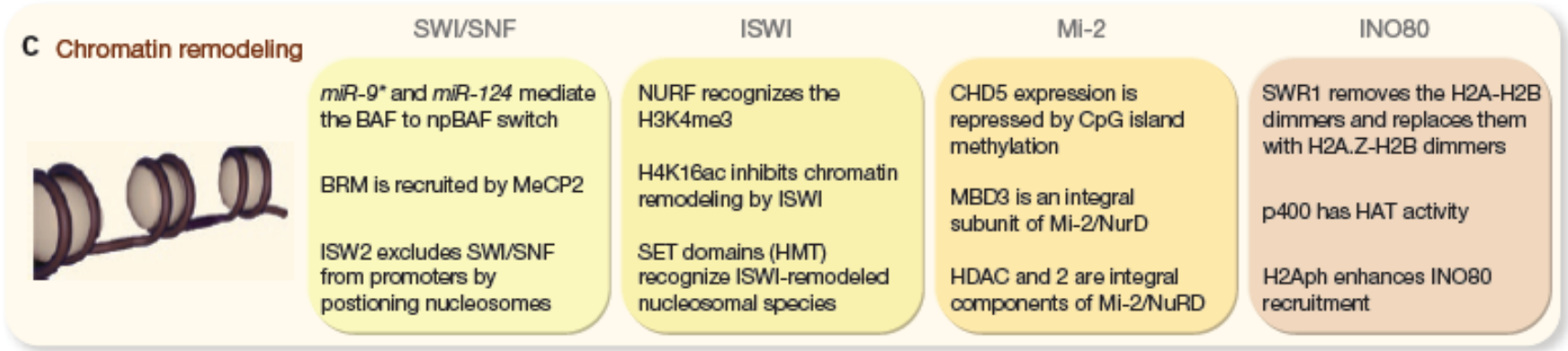
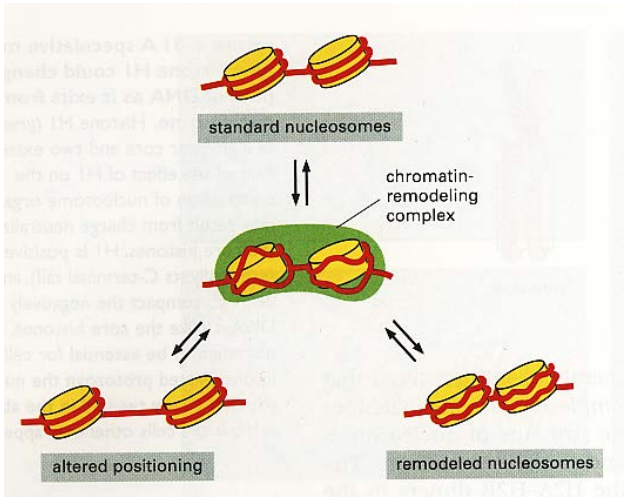
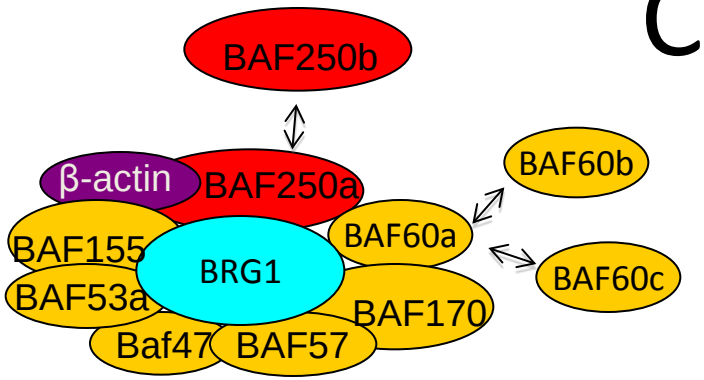
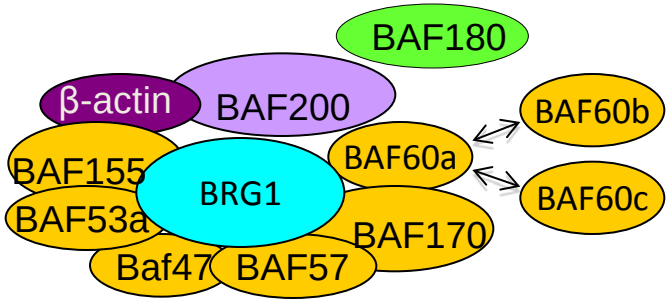


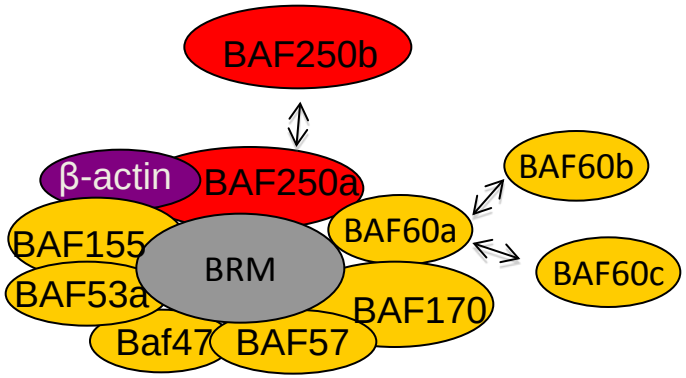
Figure 2 Epigenetic machinery and interplay among epigenetic factors. Epigenetic marks are catalyzed by different epigenetic complexes, whose principal families are illustrated here. (**a–c**) Epigenetic regulation depends on the interplay among the different players: DNA methylation (**a**), histone marks (**b**) and nucleosome positioning (**c**). The interaction among the different factors brings about the final outcome. This figure illustrates selected examples of the possible interrelations among the various epigenetic players.



Components of the SWI/SNF Complex







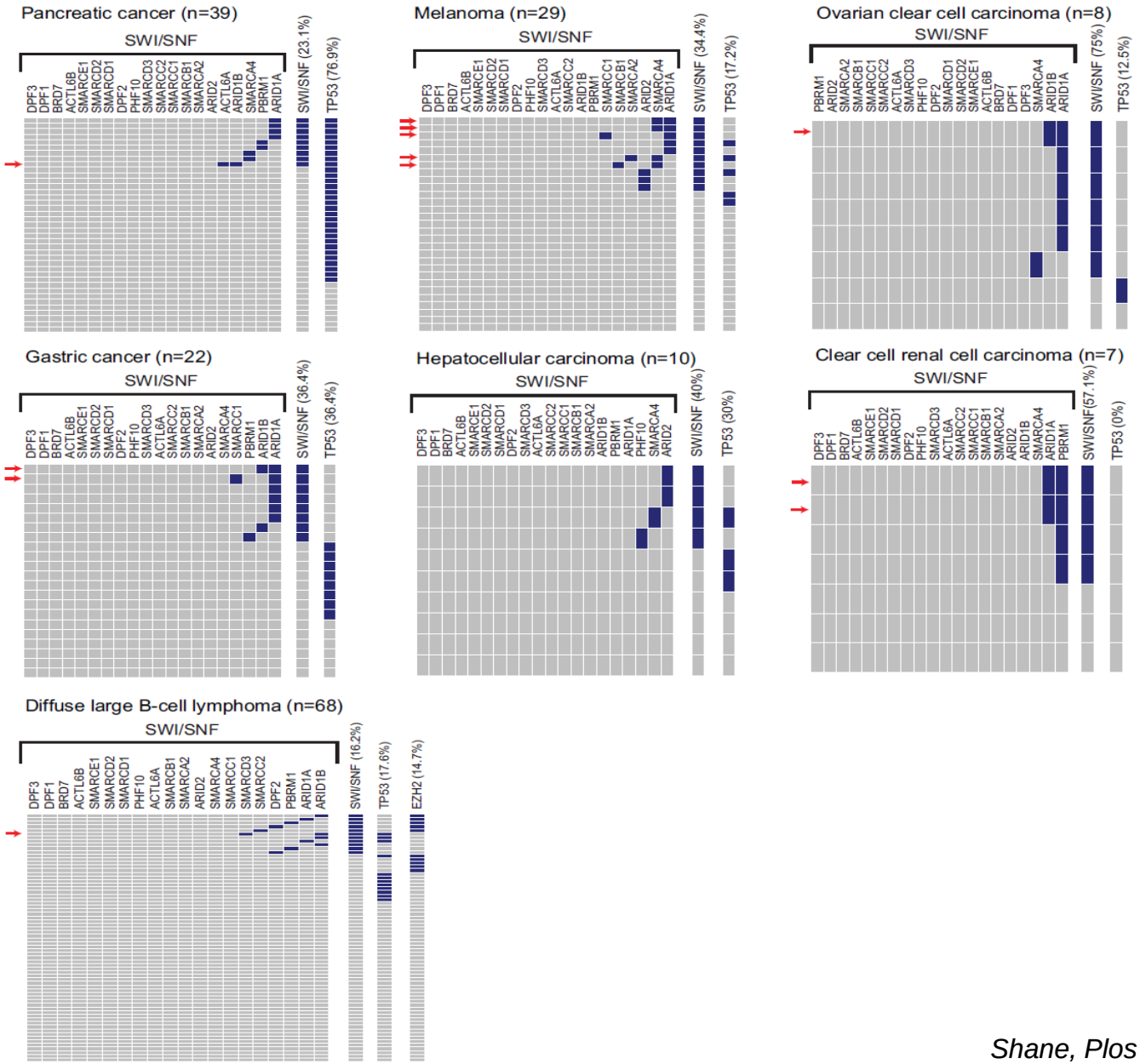
Disruption of SWI/SNF components in cancer

Table 1 | **SWI/SNF mutations in cancer**

SWI/SNF subunit	Associated cancers (mutation frequency)	Primary tumours or cell lines	Haploinsufficiency or homozygous inactivation	Types of mutations	Refs
SNF5	Rhabdoid tumours (98%)	Primary tumours and cell lines	Homozygous inactivation	Homozygous deletion, nonsense, missense and frameshift mutations	30–33
	Familial schwannomatosis (30–40%)	Primary tumours	Homozygous inactivation	Truncating mutations	34,39, 137–139
	Small-cell hepatoblastomas (36 %; 4 of 11)*‡	Primary tumours	Homozygous inactivation	Translocations and homozygous deletion of 22q11.2	35
	Extraskeletal myxoid chondrosarcomas (8%; 2 of 24)*	Primary tumours	Homozygous inactivation	Frameshift and homozygous deletion	36
	Undifferentiated sarcomas (29%; 5 of 17)*	Primary tumours	Haploinsuffiency and homozygous inactivation	Homozygous deletion and intragenic mutation	37
	Epitheliod sarcomas (55%; 6 of 11)*‡	Primary tumours	Homozygous inactivation	Homozygous deletion	38
	Meningiomas (<3%; 4 of 126). Frequency may be higher in familial meningiomas*	Primary tumours	Homozygous inactivation	Missense mutations with loss of the second allele	39, 140,141
	Poorly differentiated chordomas (3 of 4)*‡	Primary tumours	Homozygous inactivation	Loss of 22q11.2	40
BAF180	Renal cell carcinoma (41%; 92 of 227)	Primary tumours and cell lines	Homozygous inactivation	Truncating mutations (34%; 88 of 257), nonsense, missense and frameshift mutations	50
	Breast cancer	Cell lines	Homozygous inactivation	Truncating mutations	51
ARID1A	Ovarian clear cell carcinoma (50%)	Primary tumours and cell lines	Haploinsufficiency and homozygous inactivation	Truncating mutations	57,58
	Endometriod carcinoma (35%; 10 of 33)	Primary tumours and cell lines	Haploinsufficiency and homozygous inactivation	Truncating mutations	57,58
	Renal cell carcinoma	Primary tumours	Homozygous inactivation and haploinsufficiency	Homozygous deletions and heterozygous missense mutations	50
	Medulloblastoma (1 of 88)	Primary tumours	Not determined	Truncating mutations	59
	Lung cancer	Cell line	Homozygous inactivation	Intergenic deletion	60
	Breast	Primary tumour	Not determined	Genomic rearrangement	60
BRG1	Non-small-cell lung cancer (35%; 13 of 37 cell lines)	Cell lines	Homozygous inactivation	Homozygous truncating mutations and missense mutations	67
	Lung cancer (frequency unclear)	Primary tumours	Homozygous inactivation and haploinsufficiency	Missense, insertion and nonsense mutations	65, 66,70,72
	Medulloblastoma (3%; 3 of 88)	Primary tumours	Not determined	Missense mutations	59
	Pancreatic, breast and prostate	Cell lines	Homozygous inactivation and haploinsufficiency	Truncating mutations and missense mutations	71
	Rhabdoid tumours	Primary tumours	Homozygous inactivation	Truncating mutations	73
BRD7	Breast cancer‡	Primary tumours	Not determined	Genomic loss on chromosome arm 16q. Reduced expression in 20% of primary tumours	82

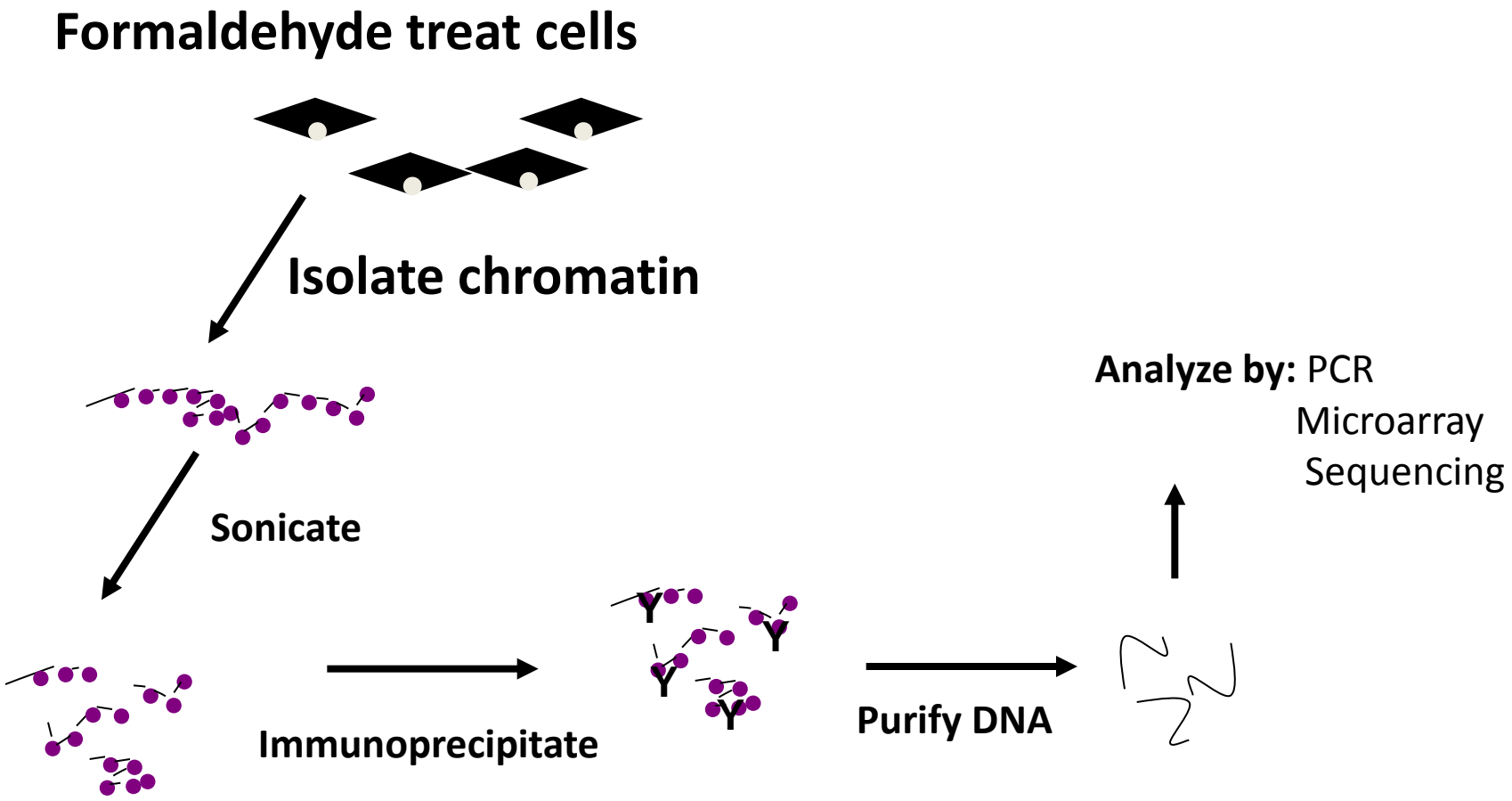
ARID1A, AT-rich interactive domain-containing protein 1A (also known as BAF250A and SMARCF1); BRD7, bromodomain-containing 7; BRG1, BRM/SWI2-related gene 1 (also known as SMARCA4). *These cancers might represent rhabdoid tumours with an atypical histological appearance. ‡These cancers carry large multi-gene deletions rather than SNF5- or BRD7-specific mutations.

(Wilson and Roberts, Nat. Reviews 2011)



Shane, Plos One, 2012

Chromatin Immunoprecipitation for investigating histone modifications



Analysis by ChIP-Seq

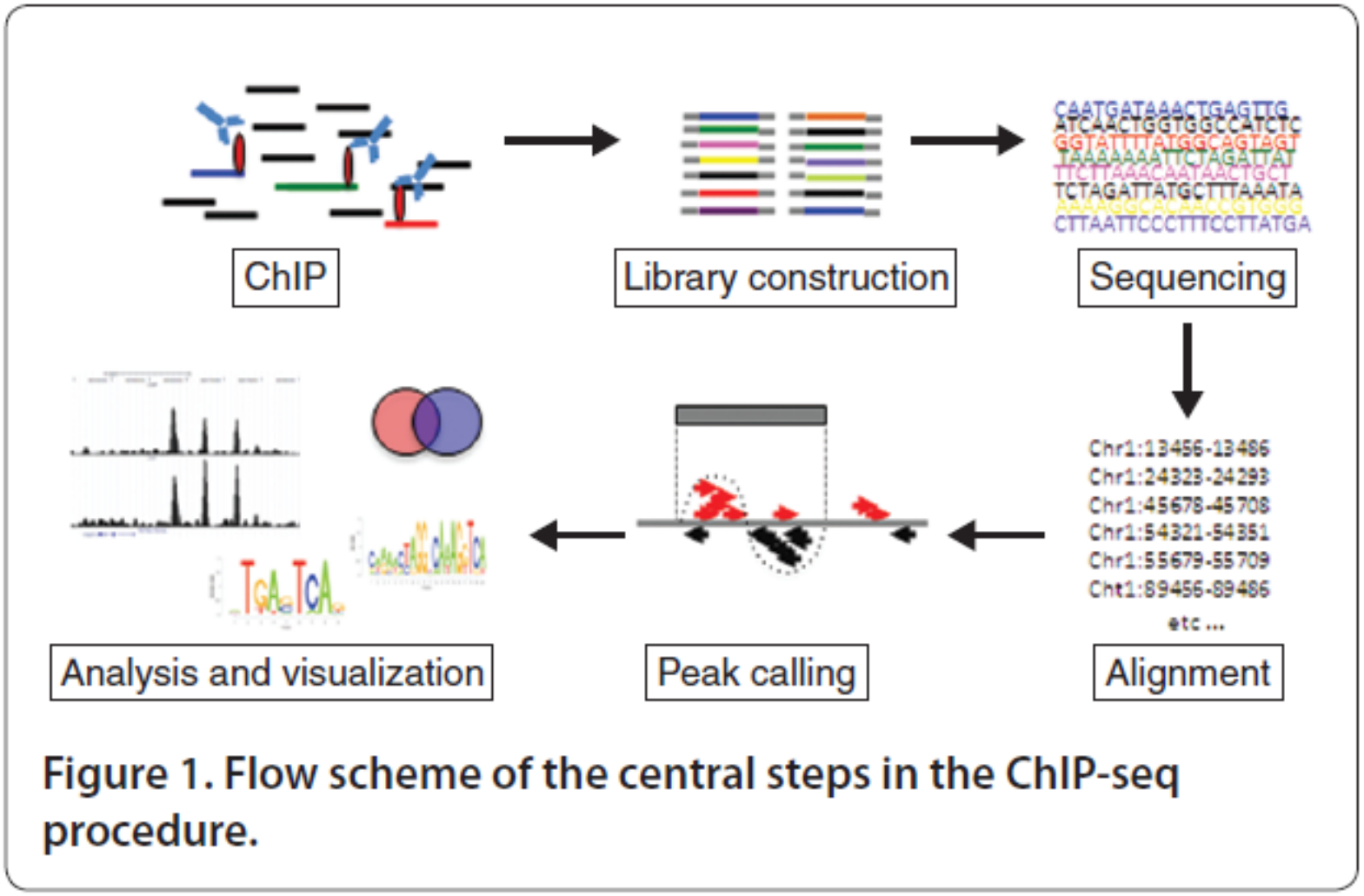


Figure 1. Flow scheme of the central steps in the ChIP-seq procedure.

Pathology tissue-chromatin immunoprecipitation (PAT-ChIP)

- Pathological specimens from surgery or biopsies are crosslinked with formaldehyde at high concentrations(4%) for an extended period of time (overnight).

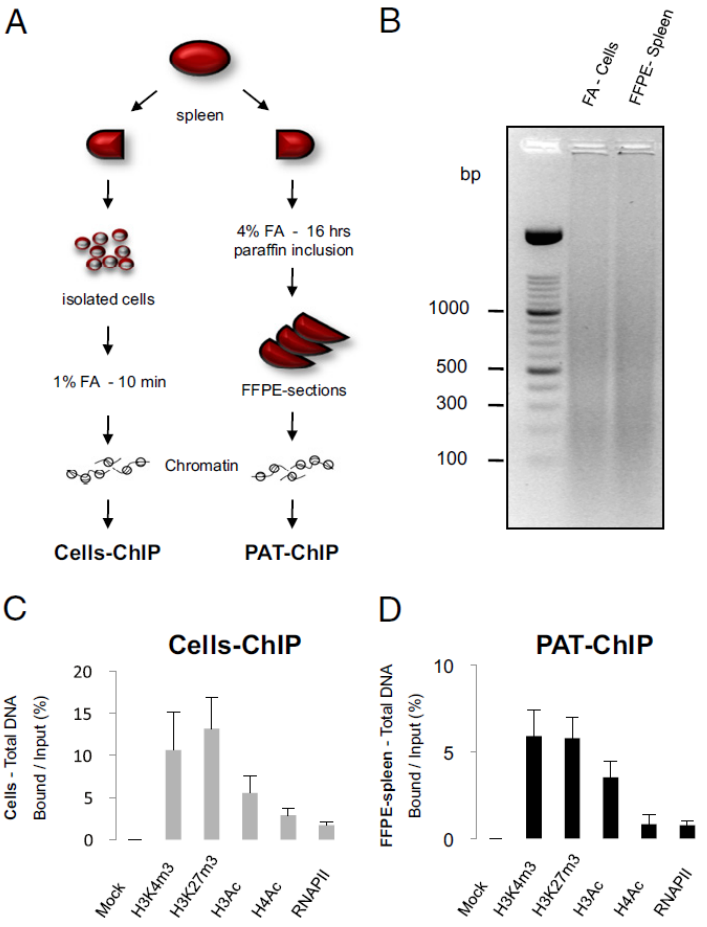
- The fixed tissues are then dehydrated and included in paraffin

PAT-ChIP

- Deparaffination of tissue samples by sequential incubations in histolemon solution
- Rehydration by decreasing concentrations of ethanol from 100% to 95%, 70%, 50%, and 20%, with water as a final step
- Isolate chromatin and perform ChIP assay

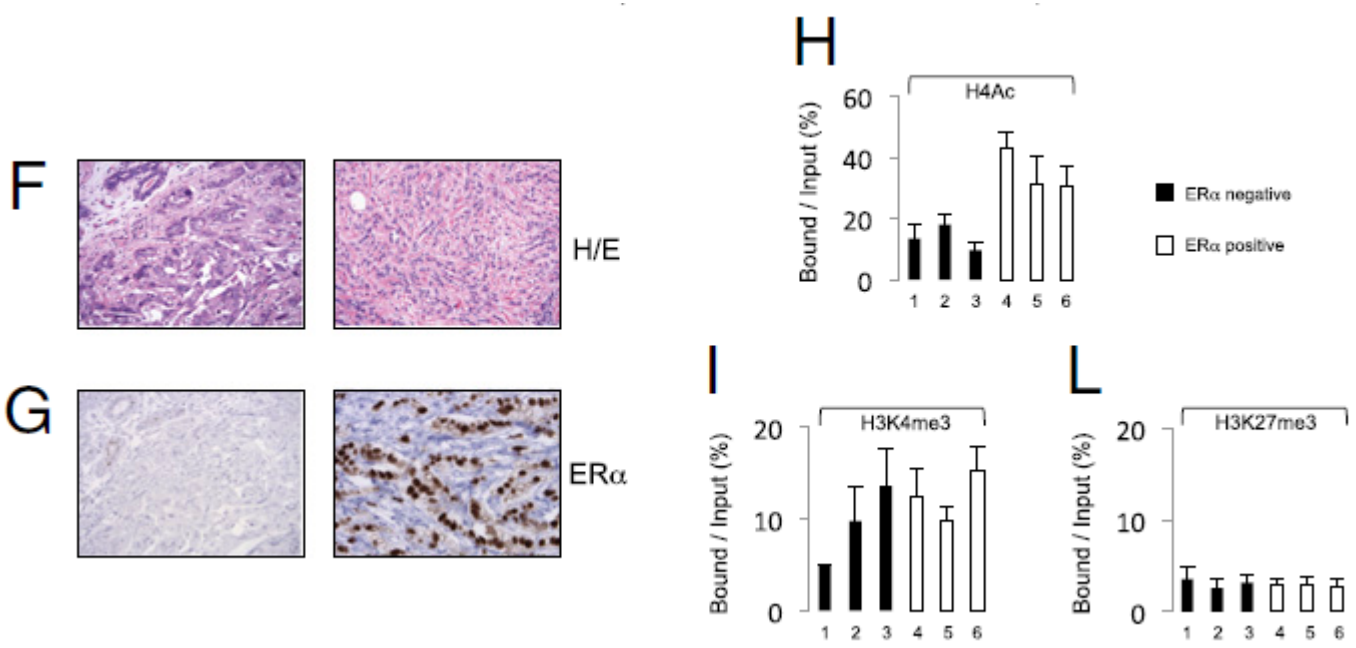
Fanelli et al PNAS 2010

Comparison of Cells-ChIPs vs PAT-ChIP



Fanelli et al PNAS 2010
Detailed protocol Fanelli et al, Nature Protocols, 2011

Use of PAT-ChIP in Human Specimen



Potential Epigenetic Biomarkers

Expression/mutational status of histone modifying enzymes

PRC components: EZH2, BM1

SWI/SNF components: BAF47, BRG1, BRM

DNA Methylation /hydroxymethylation

Changes at specific loci or regions may be indicative of disease

Changes in genomic levels

Histone covalent modifications

Changes at specific loci or regions may be indicative of disease

Changes in genomic levels:

Decreased H3K16ac, H3K4me3, H4K20me3, increased H3K9me

Epigenetic Treatment

- HDAC Inhibitors: target the catalytic domain of HDACs, thus interfering with their substrate recognition

Short chain fatty acids:

Sodium phenylbutyrate, sodium butyrate, and valproic acid

Hydroxamic acids:

Trichostatin A, vorinostat, and panobinostat

Cyclic Peptides:

Romidepsin

Benzamides:

MGCD-0103

eninostat

HDACis are used in conjunction with other drugs

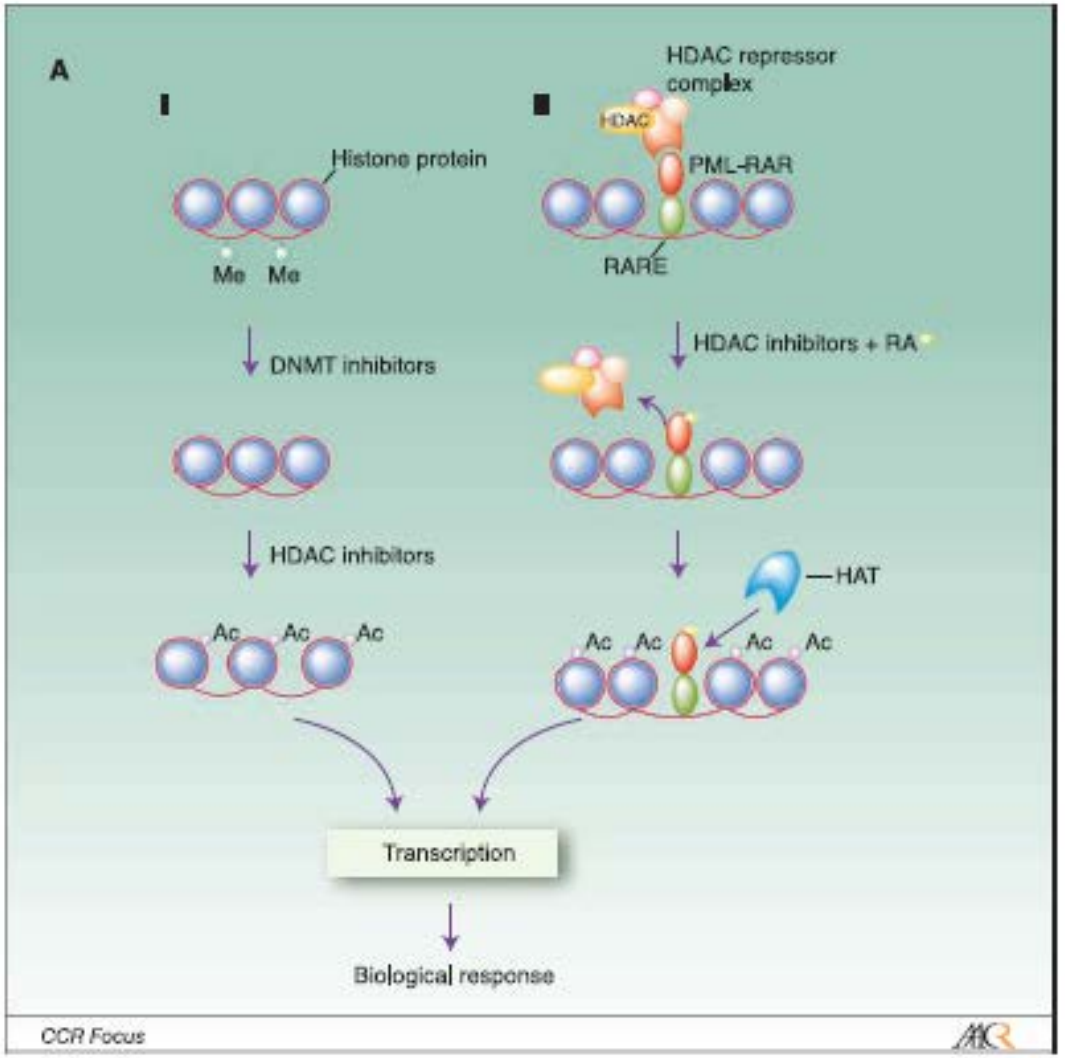
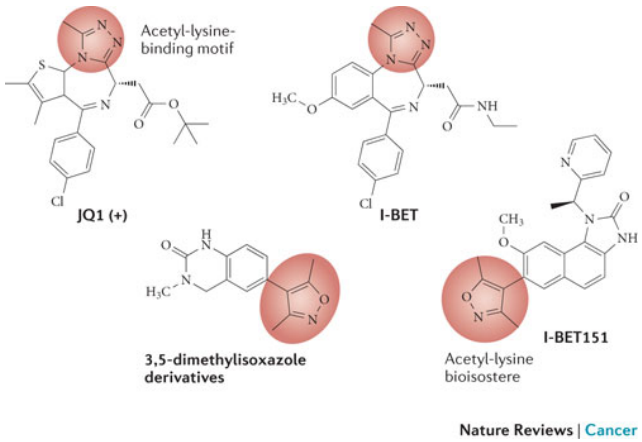
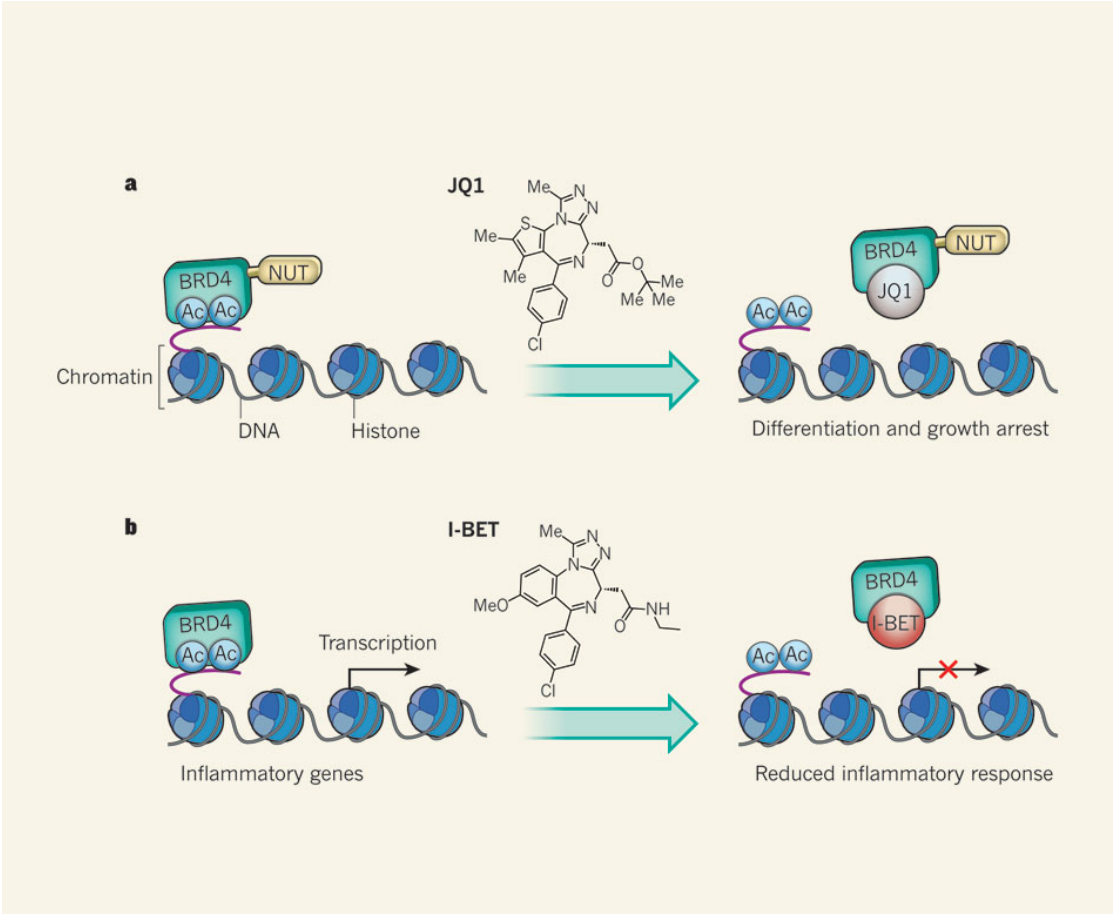


Fig. 1. HDAC inhibitors act synergistically with anticancer drugs by various mechanisms. *A*, combination strategies acting via transcription. *i*, hypermethylation of genes is an often-observed mechanism to silence transcription of target genes. DNA demethylation using DNMT inhibitors in combination with hyperacetylation of histone proteins results in a more "open" chromatin structure mediating enhanced gene transcription. *ii*, PML-RARα bound to a retinoic acid response element (RARE) recruits an HDAC-containing repressor complex resulting in transcriptional repression. HDAC inhibitors and retinoic acid (RA) induce dissociation of the repressor complex, recruitment of coactivators with histone acetyltransferase (HAT) activity, increased levels of histone acetylation, chromatin remodeling, and transcriptional activation.

Inhibiting BET-family proteins





Summary

- Epigenetics: “An epigenetic trait is a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence.
- The best characterized epigenetic changes to occur in human diseases involve changes in DNA methylation profiles and/or histone modifications.
- These changes are amenable to therapeutic intervention.

Biomarkers are characteristics which are objectively measured and evaluated as an indicator of the intrinsic causes of illnesses, the clinical course, and its modification by treatment.

1. Gene expression
2. Mutation analysis
3. DNA modifications
4. Chromatin modifications

References

- Portella and Esteller (2010) Epigenetic Modifications and Human Disease.Nature Biotech 28:1057-1068
- Taby and Issa (2010) Cancer Epigenetics. CA CANCER J CLIN 2010;60:376–392
- Allis and Muir (2010) Spreading Chromatin into Chemical Biology. ChemBiochem. 12, 264 – 279
- Required readings: hand outs