

EPIGENETICS

1. Definitions

Epigenetic: Clonal changes in gene expression that do not involve changes in the coding region of affected genes.

Silencing: A clonal, usually irreversible suppression of gene expression that can be transcriptional or post-transcriptional.

2. Why epigenetics? Physiologic functions

Development: Epigenetics are thought to play an important role during early embryonic development. In general, severe epigenetic defects are embryonically lethal, and incomplete epigenetic resetting contributes to the low success of cloning animals from adult cells. The exact pathways affected are poorly understood.

Differentiation: Differentiation is epigenetic in some cases, but it also involves transcriptional programs (e.g. activation of specific transcription factors in some cells but not others) rather than specific silencing. For example, the muscle tissue phenotype is primarily related to activation of muscle-specific transcription factors.

Host defense: Silencing is thought to have evolved initially as a mechanism of host defense. Bacteria use restriction enzyme digestion to eliminate foreign DNA, and methylation to protect its own genome against these enzymes. Many organisms silence foreign DNA by post-transcriptional mechanisms. In mammals, integrated parasitic DNA is usually heavily methylated and silenced. (In humans, Alu, LINE and retroviral elements represent a large portion of the genome and are silenced epigenetically).

Sex chromosome gene expression: Organisms that use sexual reproduction have evolved mechanisms to equalize expression of genes on the sex chromosomes. In mammals, females have two X-chromosomes while males have only one. To achieve equal gene expression, one copy of the X-chromosome is usually silenced in females.

Imprinting: Imprinting refers to parentally-determined monoallelic gene expression, a mechanism that is thought to affect 100-200 genes in humans and that is currently well described only in mammals. For example, the IGF2 gene main promoters are expressed only from the paternally derived allele, while the nearby H19 gene is expressed only from the maternally derived allele. Thus, one allele is silenced in each case.

Chromosome structure: Centromeres usually have a high concentration of repeated DNA, including both common repeats and satellite DNA. Some of these are potentially transcriptionally active. Silencing of this transcription is likely important for normal chromosome condensation and segregation. Similarly, telomere areas often have repeated elements and these are frequent sites of silencing.

3. Molecular mechanisms of epigenetics

3.1. Mutational – RIP or Repeat-Induced Point Mutations (Neurospora)

An unusual process whereby repeated genes/foreign DNA is targeted for mutational inactivation, with potentially hundreds of mutations arising rapidly after the gene duplication event. The DNA is also hypermethylated/silenced, but the two processes are probably unlinked. This is well described only in rare instances (Neurospora Crassa).

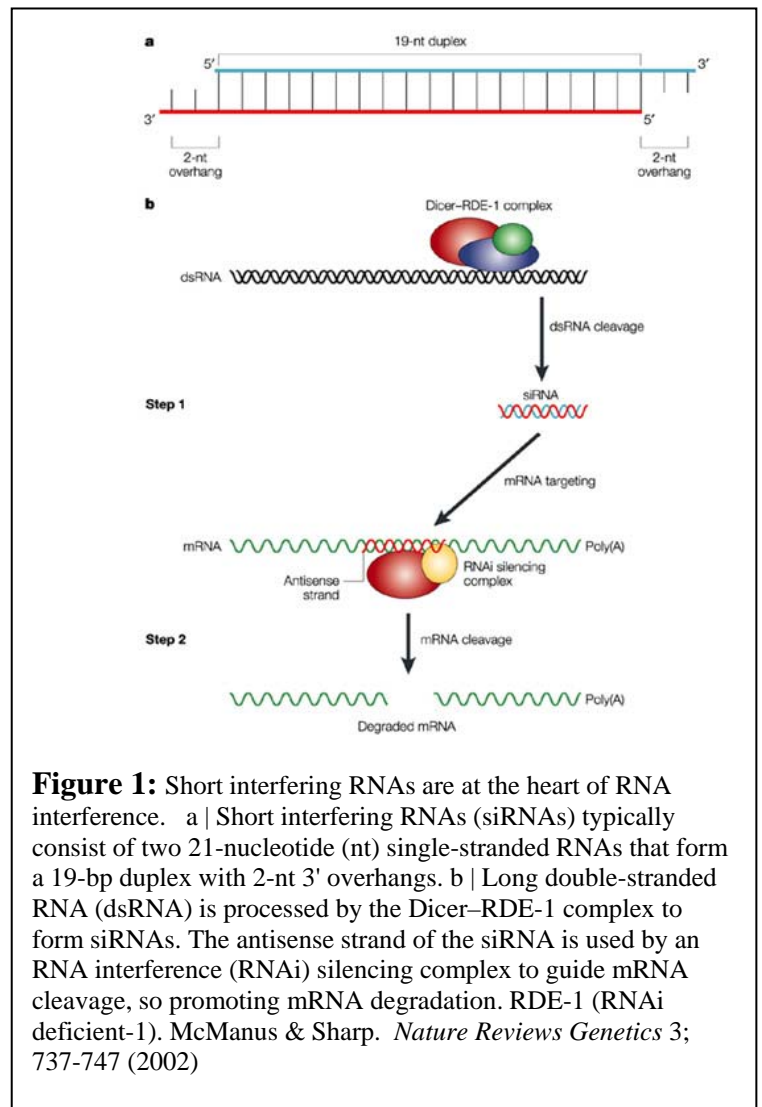
3.2. Post-transcriptional (PTGS)

Can be either mRNA degradation, translational block or protein degradation. Only mRNA degradation is currently well-described as a mechanism of silencing.

Co-suppression: A biological phenomenon whereby a transgene results in silencing of both the inserted gene and the endogenous gene. Caused by RNAi.

RNAi or RNA interference: A mechanism of rapid mRNA degradation targeted to specific (silenced genes). Initially, double-stranded RNA triggers the process that is then amplified and maintained by specific mechanisms. Eventually, short RNA molecules complementary to the silenced gene bind the native RNA and target RNAses to that molecule (Figure 1). The process is so efficient that it is frequently used in the lab to down-regulate gene expression and probe for function. It may even work as a gene therapy process. In Yeast and plants, RNAi plays a role in silencing retrotransposons, and is important for chromosomal structure. Mammalian cells are capable of RNAi but physiologic functions in that setting are unknown.

MicroRNAs: Mammalian genomes transcribe small RNA molecules that regulate gene expression through mRNA stability. These microRNAs are part of normal gene regulation and are distinct from epigenetic processes such as RNAi, although they use the same machinery in some cases (e.g. Dicer). The main difference between microRNAs and RNAi may be related to the fact that microRNAs have 1 (or more) bases mismatched compared to the target molecule, making the process of RNA degradation less efficient.



Transcription elongation: DNA methylation was reported to inhibit transcript elongation in Neurospora, but this has not been described in other organisms.

3.3. Transcription initiation suppression

Transcriptional silencing is a conserved physiologic mechanism throughout evolution. Silencing (irreversible) is mediated by a closed chromatin configuration that excludes transcription factors. Thus, genes are not expressed despite the continued presence of their transcription factors. Chromatin is packaged by histone proteins (Figure 2), and modifications of these histones determine the tightness of packaging (hence the degree of silencing).

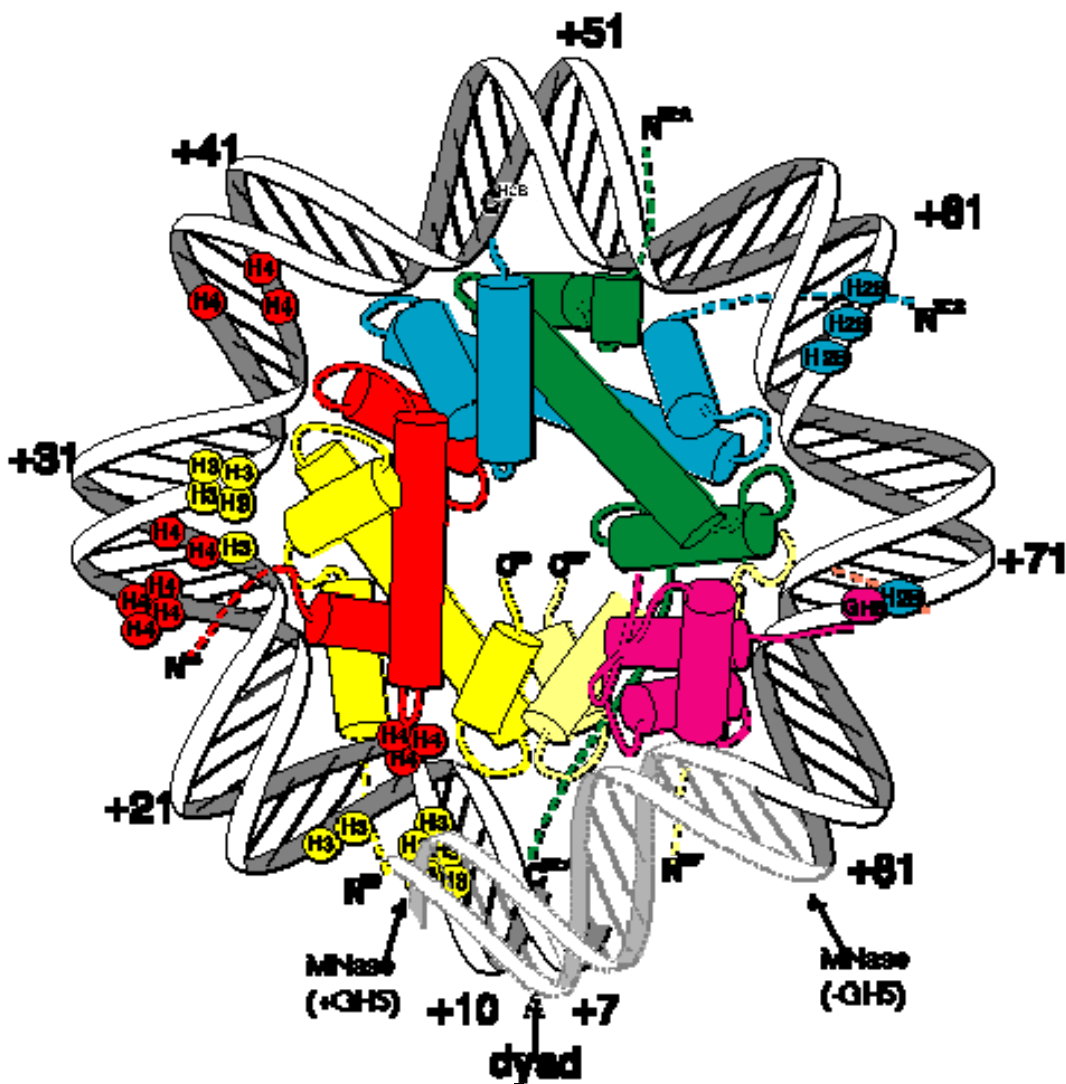


Figure 2: The nucleosome unit, from <http://www.average.org/~pruss/nucleosome.html>

3.3.1. The histone code

Jenuwein and Ellis proposed that the various histone tail modifications form a code that determines the eventual state of gene expression (see the review and figures 3&4). There are 5 described histone modifications that could play a role there:

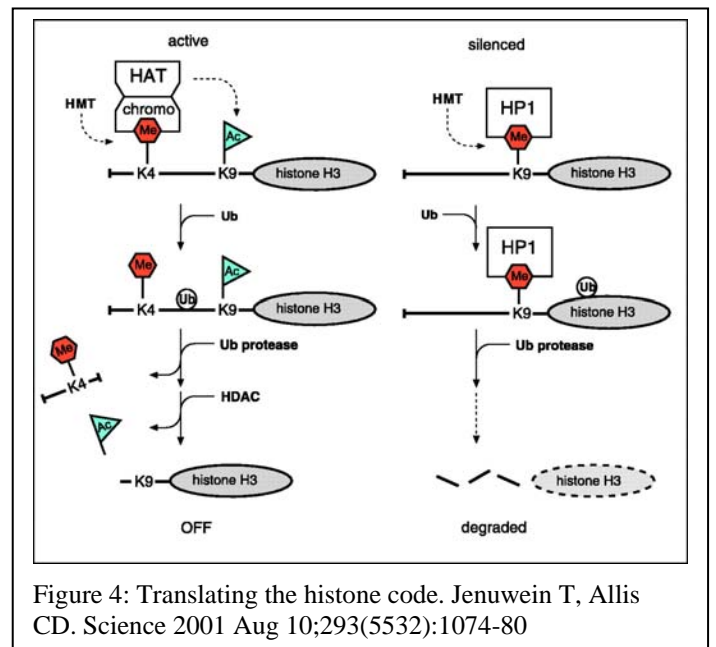
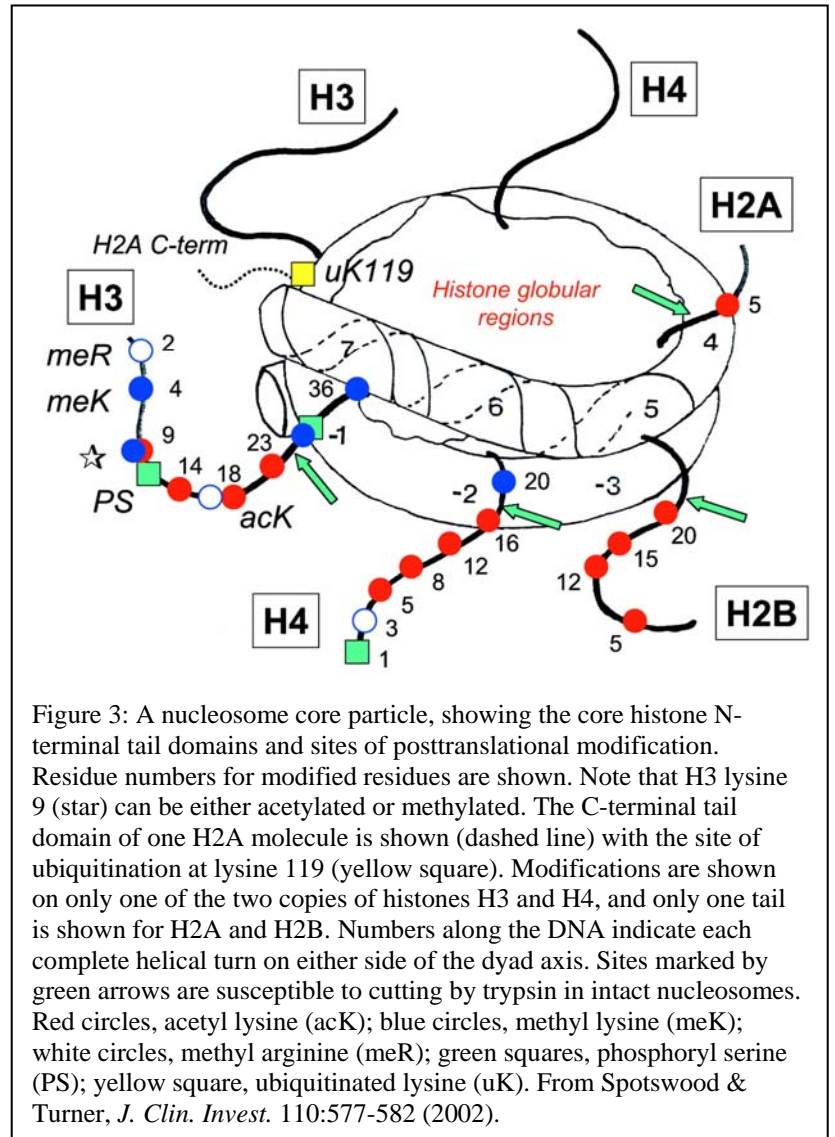
Acetylation/deacetylation: In general acetylation is associated with expression while deacetylation is associated with reversible lack of expression there are many histone acetylases (e.g. CBP in mammals) and histone deacetylases (HDAC1-11).

Methylation/demethylation: Histone methylation (not to be confused with DNA methylation) can be associated with either active genes (e.g. lysine 4 methylation), or silenced genes (e.g. lysine 9 methylation). Lysine 9 appears to be a central modification of H3, with acetylation resulting in readiness for expression and methylation (which is mutually exclusive with acetylation) resulting in silencing. Lysine 27 is another critical modification for gene silencing and its methylation is the first step in X-inactivation in mammals. Several histone demethylases were recently described; thus methylation/demethylation can serve as a switch in the same way acetylation/deacetylation does.

Phosphorylation, Ubiquination and ADP

Ribosylation: Other modifications that may play a role in either nucleosome structure or silencing. Their role is not yet known in detail.

Recent data implicate the histone code and modifying enzymes in the stem cell phenotype, and in the switch from non-committed to committed (differentiated cell). In particular, genes involved in differentiation are found in a “poised” state in ES cells, where they have histone marks characteristic of active genes (H3K4 methylation) and, simultaneously, inactive marks (H3K27 trimethylation and PcG occupancy). Commitment involves switching from poised to fixed (either active or inactive).



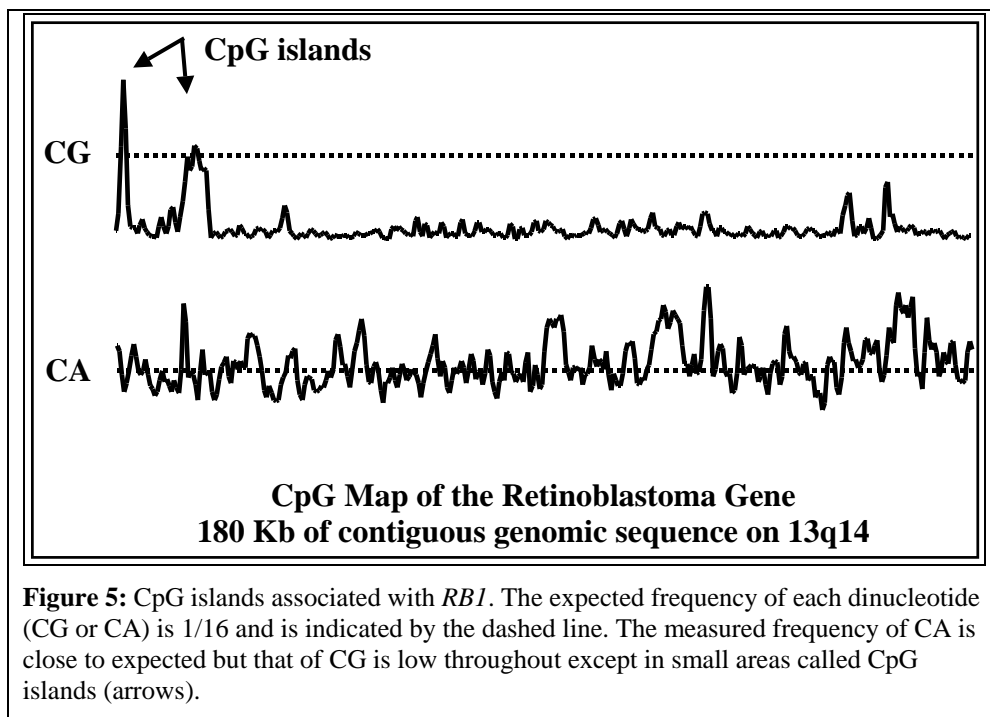
3.3.2. DNA methylation

Methylation: A biochemical modification that consists of the addition of a methyl group – can affect proteins, RNA or DNA.

DNA Methylation: In mammals, only affects Cytosine, primarily as part of the CG (CpG) dinucleotide. Methylation is a post-DNA synthesis modification that is involved in silencing. The methylated C is hypermutable (on an evolutionary scale), through deamination.

CpG Suppression: In mammals, the frequency of CpG is only 10-20% of expected, because of methylated C mutations (to T) (over the millions of years of evolution).

CpG islands: Small (.3-2kb) regions of DNA where the CpG density is as expected or higher. Usually defined based on overall C&G content (>50%), CpG content (>5%) and CpG/GpC ratio (≥ 0.6). CpG islands are usually associated with genes. About 50% of genes have CpG islands in their promoter region.



Normal DNA methylation patterns

CpG islands (~1% of DNA) and non-CpG island regions have different patterns of methylation in normal tissues. Methylation patterns are established early during embryogenesis. Initially, a wave of demethylation erases nearly all methylation from DNA. After that, de-novo methylation occurs, establishing adult patterns of methylation. They consist of (1) complete lack of any methylation within most CpG islands (exceptions are discussed below) and (2) 80% methylation of non-island CpG sites. Methylated cytosines are particularly common in peri-centromeric satellite DNA where it contributes to chromosomal stability, as well as within DNA repeats (Alus, LINEs), where it has been proposed to serve as a 'defense mechanism' against inappropriate expression and recombination within these repeats.

One recognizes then 4 distinct patterns of methylation:

- Non-promoter, non-CpG island DNA: Normally methylated
- Promoter, non-CpG island DNA: methylated if the gene is inactive. Demethylates rapidly when the gene is activated. Methylation does not silence the gene, but it may regulate the degree of gene activation
- Non-promoter CpG island. Usually unmethylated, but can occasionally be methylated in normal tissues. Function of these islands and consequences of methylation unknown.
- Promoter CpG island. Normally unmethylated, regardless of expression state of the gene. Exceptions are normally silenced genes (e.g. inactive-X chromosome).

After these dramatic changes in methylation during embryogenesis, there are relatively few (and very slow) additional redistribution of methylation patterns during development and aging.

The methylation machinery

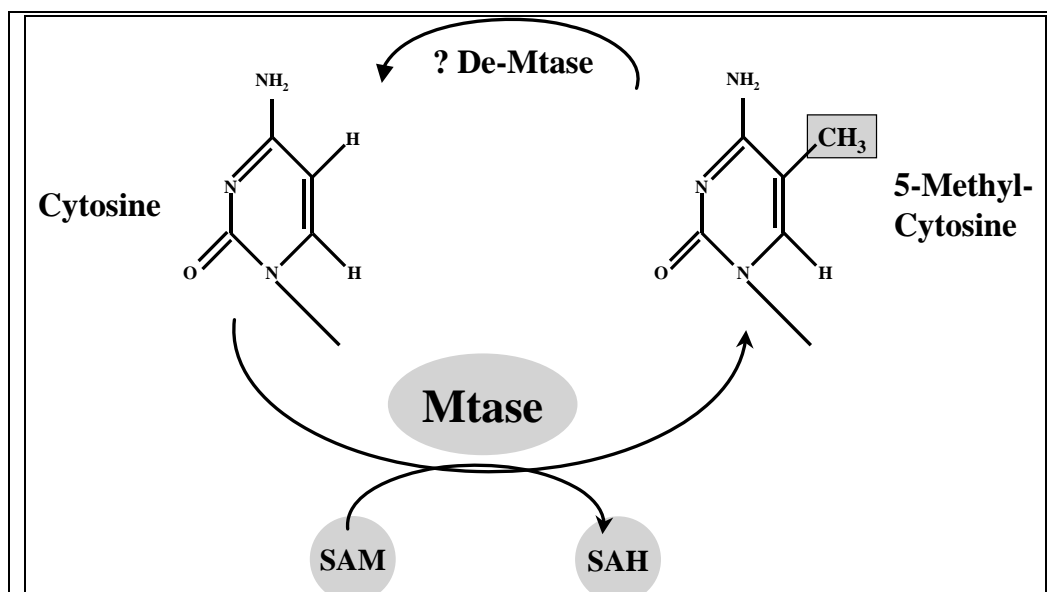


Figure 6: DNA methylation (in humans) simply consists of the addition of a methyl group to the 5 position of cytosine. This is done post-replicatively by a group of enzymes called DNA-methyltransferases (Mtase), with S-adenosyl-methionine (SAM) serving as methyl donor. A putative demethylase has recently been described that is purported to catalyze the reverse reaction. The existence of this enzyme remains controversial.

DNA-methyltransferases: There are three described Mtases (Figure 6). *DNMT3a* and *DNMT3b* are de-novo methylases whose function is to methylate previously unmethylated DNA. They are primarily active during embryogenesis. Knock-out of both enzymes is embryonically lethal. The rare disease ICF is caused by mutations in *DNMT3b*. By contrast, *DNMT1* is a ‘maintenance’ methylase. That is, its primary function is to methylate DNA post-replicatively, at which point DNA is methylated on only one strand (hemi-methylated). *DNMT1* rapidly methylates the newly synthesized strands at all CpG sites where it encounters hemi-methylation, thus faithfully reproducing embryonic methylation patterns (Figure 7). CpG sites that start out unmethylated (in CpG islands, for example) do not get methylated by *DNMT1*. Knock-out of *DNMT1* is embryonically lethal.

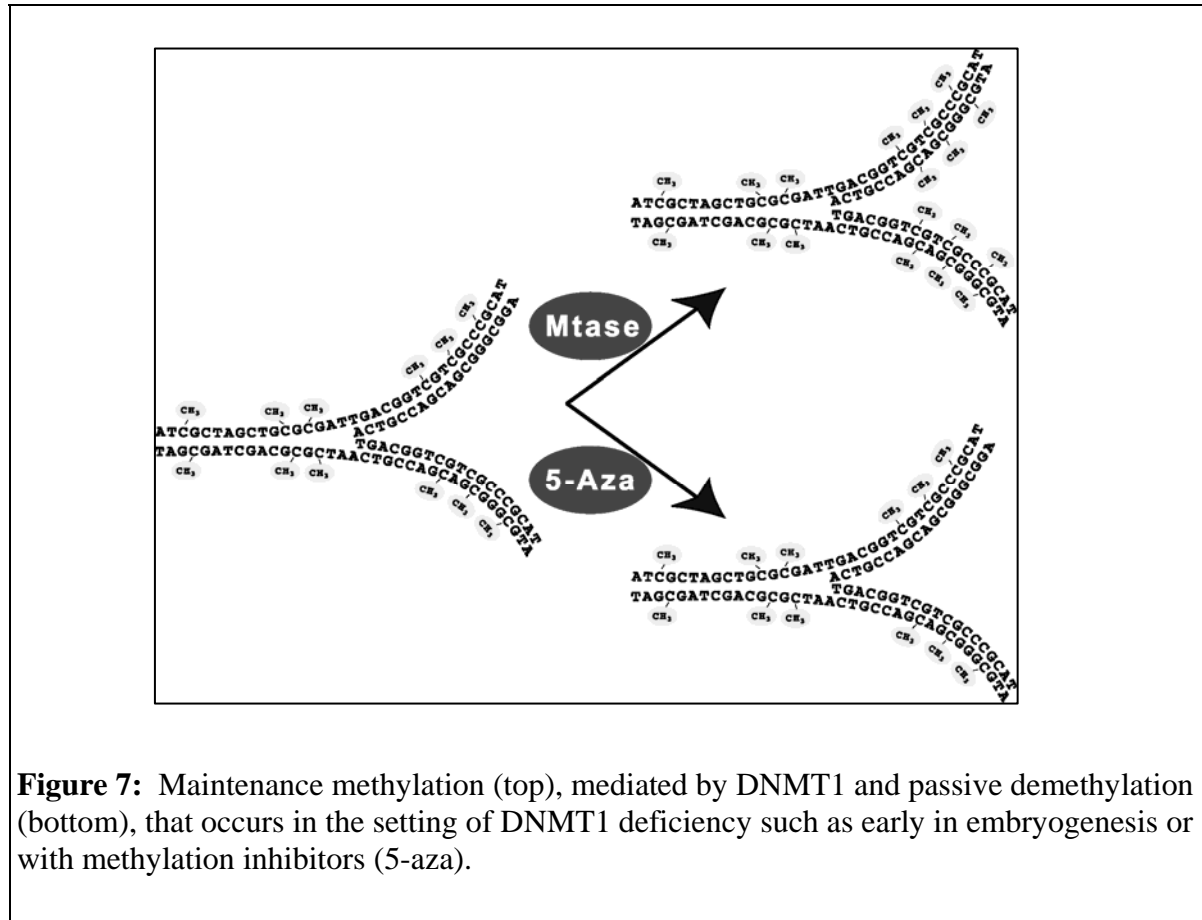


Figure 7: Maintenance methylation (top), mediated by DNMT1 and passive demethylation (bottom), that occurs in the setting of DNMT1 deficiency such as early in embryogenesis or with methylation inhibitors (5-aza).

Demethylation/demethylases: Demethylation occurs primarily when Mtase levels are low. In this situation, newly synthesized DNA does not get remethylated, and after a few rounds of replication, the DNA becomes largely unmethylated. This is referred to as ‘passive demethylation’. ‘Active’ demethylation has been described but remains a controversial issue.

Methyl-binding proteins: There is a family of proteins that bind methylated DNA with high affinity. Examples are *MeCp2* and *MBD1-4*. Some of these proteins have transcriptional repression domains. In addition, some of these associate with protein complexes that contain histone deacetylases. The interaction of methylation/methyl-binding proteins is thought to be essential to the normal function of DNA methylation. *MeCp2* mutations cause the RETT syndrome.

Methylation centers and protectors: One model of the formation of methylation patterns is that methylation originates in methylation ‘centers’ (perhaps repeats such as Alus) that attract Mtase, and spreads in cis from there both upstream and downstream. CpG islands, however, resist this spreading through the action of ‘protectors’, proteins that bind the island and stop Mtase from methylating. Much of the details of this model remain sketchy.

CpG island methylation as a mechanism of epigenetic silencing

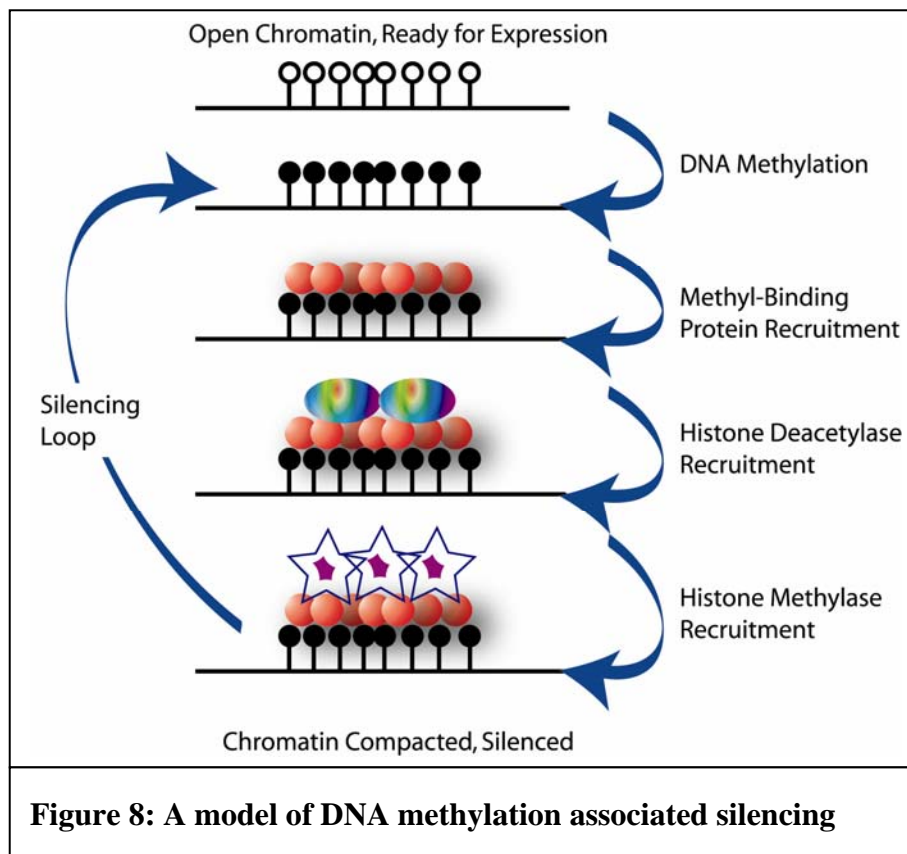
The idea that CpG island methylation could be associated with transcriptional suppression originated in the early 80’s, when it was demonstrated that genes on the inactive X-chromosome in women were characterized

by such methylation. Soon thereafter, a similar differential methylation was described for imprinted genes. Evidence that methylation directly contributes to transcriptional suppression came from the following experiments:

- (1) The cells under study were found to be capable of expressing an exogenous unmethylated construct but had little or no expression of methylated constructs.
- (2) The use of pharmacologic inhibitors of DNA methylation resulted in reexpression of the silent alleles.
- (3) Mouse cells where *DNMT1* is deleted have loss of imprinting and X-inactivation.

In both X-inactivation and imprinting, methylation may not be the primary triggering event for silencing, but it is required for maintaining the silenced state.

The mechanism of transcriptional silencing by DNA methylation is beginning to be understood. Our current understanding of the process is that a cascade of events is initiated by DNA methylation, once a 'threshold' of methylation density (number of CpG sites methylated on a given allele) is reached. This cascade involves the (1) recruitment of methyl-binding proteins, (2) recruitment/targeting of histone deacetylases to the affected promoter, (3) recruitment/targeting of histone lysine 9 and 27 methylase(s) all of which result in chromatin compaction into a state that does not allow access of the DNA to transcription factors. This state is maintained as long as DNA methylation persists. Other contributing factors to silencing are (1) direct inhibition of transcription factor binding by methylation of its binding site (in some cases), (2) direct recruitment of histone deacetylase by *DNMT1* and (3) direct transcription suppression by MeCp2. New data indicates that, in some instances, histone lysine 9 methylation itself recruits DNA methylation to a given promoter. Thus, there is a silencing loop that explains why this process is so stable.



4. Epigenetic diseases

A number of diseases (primarily developmental) have now been shown to have a major epigenetic component

Rett syndrome: An inherited neuro-degenerative disease in young girls that is caused by mutations in MeCP2.

Fragile X disease: An inherited mental retardation syndrome in young boys caused by expansion of a CGG repeat in the FMR1 gene followed by methylation and silencing of the gene.

ICF syndrome: Immunodeficiency, Centromeric instability and Facial anomalies – a very rare inherited disease caused by mutations in DNMT3B.

ATRX syndrome: Thalassemia of unclear cause. Methylation changes have been described in affected patients.

Imprinting diseases (Prader-Willi/Angelman, Beckwith-Wiedeman): A series of diseases caused by abnormal expression of imprinted genes.

5. Epigenetic Defects in Cancer

Given that normal cells have built-in mechanisms for gene silencing, it is not surprising that cancer cells acquire the ability to hijack this system to silence undesirable genes (such as tumor-suppressor genes) or activate desirable genes (oncogenes). Epigenetic defects in cancer include:

- DNA methylation abnormalities
- HDAC recruitment by fusion genes
- Translocations involving Histone Acetyl Transferases
- Translocations involving Histone Methylases
- Abnormalities of global chromatin regulators
- Imprinting defects

5.1. DNA methylation changes in cancer cells

There are complex changes in DNA methylation in cancer. For the most part, these changes involve simultaneous global demethylation, increased Mtase expression and de-novo methylation at previously unmethylated CpG islands.

5.1.1. Hypomethylation: Demethylation was first discovered by studying overall 5-mC content in tumors, and appears to involve primarily satellite DNA, repetitive sequences and CpG sites located in introns. The cause of this demethylation remains unclear, although it could be related to alterations in proliferation or cell-cycle control. The functional consequences of hypomethylation are equally unclear. Initial suggestions that gene-specific hypomethylation can cause increased oncogene expression have, for the most part, not been confirmed experimentally. An increased mutation rate was demonstrated in cells in which severe hypomethylation (>75%) was achieved by homozygous deletion of DNMT1, a major DNA-methyltransferase enzyme, but it is not clear whether this degree of hypomethylation is ever achieved in neoplasms.

5.1.2. Increased Mtase levels: Increased enzymatic Mtase activity is a property of nearly all transformed cells. Increased mRNA levels for DNMT1, DNMT3a and DNMT3b have also been described in

some neoplasms, and these three Mtase genes probably account for the observed increase in activity. The causes and functional significance of these increases remain unclear. All Mtases appear to be cell-cycle regulated, and it has been argued that Mtase levels do in fact reflect the physiologic state of increased proliferation in neoplasia. On the other hand, DNMT1 has been reported to increase following oncogene activation, and it is possible that its levels in neoplasia reflect the various molecular defects seen in tumors. The functional significance of increased Mtase activity is also poorly defined. In several systems, increased Mtase activity has been found to be transforming, but it is not clear whether this is due to increased CpG island methylation and tumor-suppressor gene silencing, or due to direct effects on the cell-cycle. The facts that, in primary tumors and cell lines, no correlation was found between Mtase activity and gene silencing, and that Mtase-related transformation is reversible when the oncogenic stimulus is removed support the latter possibility.

5.1.3. CpG island methylation as an alternative to mutations and deletions in TSG inactivation

In parallel to global hypomethylation and increased Mtase activity, there also are distinct and frequent localized increases in methylation, often involving CpG islands. Because CpG island methylation is associated with repressed transcription that is stably inherited through mitosis, this de-novo methylation in transformed cells has been proposed to serve as an alternate mechanism for inactivating tumor-suppressor genes. Indeed, several genes have now been shown to be transcriptionally silent in neoplasia, in association with CpG island methylation. As in X-inactivation, evidence for a role of methylation in silencing comes from the facts that (1) the cells are transcriptionally competent for the genes in question and (2) demethylation restores expression and, in some cases, function. Importantly, genes whose CpG islands are hypermethylated do not have concomitant promoter or coding region mutations.

For example, in colorectal cancer, the HCT116 cell line carries one mutated unmethylated *CDKN2A* allele, while the second allele in this cell line is unmutated but densely hypermethylated and transcriptionally silent. Moreover, the *hMLH1* mismatch repair gene is unmutated but densely methylated in the mismatch repair deficient cell line RKO, and nearly normal levels of mismatch repair can be restored in this cell line by inhibiting Mtase activity using the cytidine analog 5-aza-deoxycytidine.

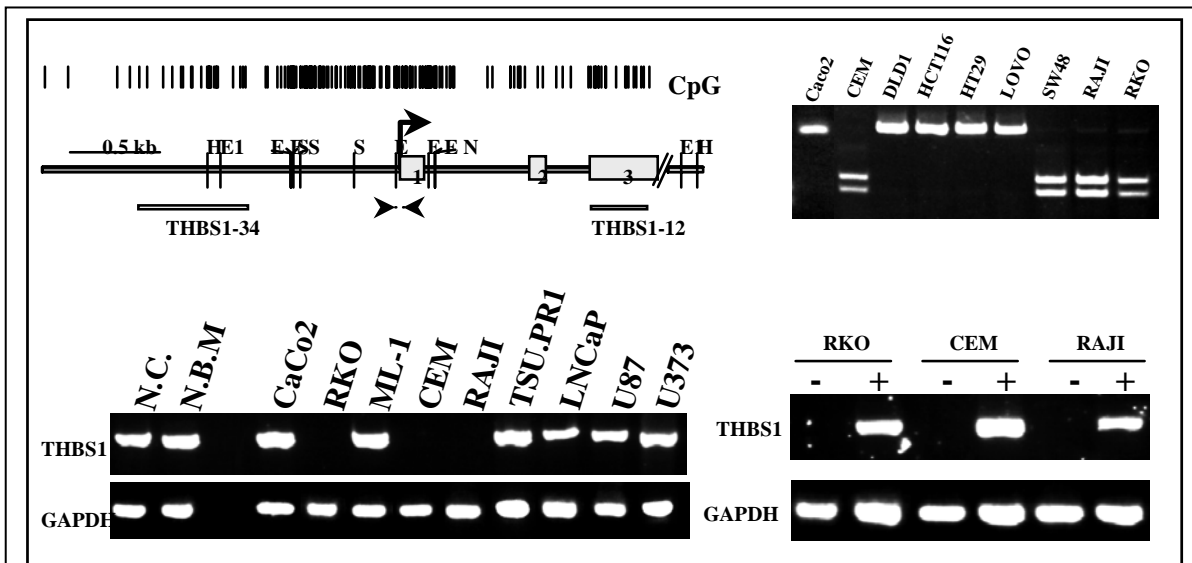


Figure 9 Example of methylation/silencing in cancer: The THBS1 gene (an inhibitor of angiogenesis). THBS1 has a typical CpG island around its first exon (top left). This island is not methylated in normal but hypermethylated in some cancer cells (top right), arrow. When hypermethylation occurs, the gene is not expressed by RT-PCR (bottom, left). Demethylation using Mtase inhibitors restores expression (bottom right, + lanes).

There is a growing number of genes that have now been shown to be hypermethylated and silenced in various cancers (Table 2). These include genes involved in cell-cycle control (CDKN2a), growth and differentiation (ER), angiogenesis (THBS1), adhesion and metastasis (TIMP3), DNA repair (MGMT), as well as others (Table 2). In addition, several genes whose function is not directly related to the cancer phenotype are also hypermethylated in some cancers. There are even examples of oncogenes hypermethylated in selected tumors. Therefore, unless there is compelling evidence for tumor-suppressor gene function, it is difficult to assign a tumor-suppressor role for a gene based solely on the presence of hypermethylation in cancer.

The most convincing evidence for CpG island methylation as a true alternative to mutations in neoplasia came from studies of *RB1*, *CDKN2A*, *VHL*, *CDH1*, *hMLH1* and others. For each of these genes, the tumor-spectrum of methylation events is virtually the same as that for mutations (table 1), and there are described cases where one allele of the gene is inactivated by methylation while the other is mutated, suggesting an equivalent growth advantage for each event in neoplasia.

Gene	Familial cancer syndrome	Mutations in sporadic cancer	Methylation in sporadic cancer	Allelic exclusion
RB1	Retinoblastoma	Retinoblastomas	Retinoblastomas	Yes
MLH1	HNPCC	Colon, endometrium	Colon, endometrium	Yes
CDH1	Stomach	Stomach	Stomach, others	Yes
VHL	Von-Hippel Lindau	Kidney	Kidney	Yes
BRCA1	Breast and ovarian cancer	Rare	Breast	ND
LKB1	Peutz-Jeghers syndrome	Rare	Same as Peutz-Jeghers syndrome	ND

Table 1: A partial list of genes hypermethylated in cancer (also see www.mdanderson.org/leukemia/methylation)

Pathway	Gene	Function	Tumors affected (examples)
Cell Cycle	RB	Inhibitor of cyclin dependent kinase	Retinoblastoma
	p16INK4A	Inhibitor of cyclin dependent kinase	Colon, lung, breast
	p15INK4B	Inhibitor of cyclin dependent kinase	Leukemia, lymphoma
	p57KIP2	Inhibitor of cyclin dependent kinase	Leukemia, stomach
	p73	Cell cycle checkpoint	Leukemia, lymphoma, neuroblastoma
	CHFR	spindle checkpoint	Colon, stomach, lung, breast
Apoptosis	DAPK	Pro-apoptotic serine/threonine kinase	Lymphoma, lung, colon, stomach
	TMS1	Pro-apoptotic CARD domain family	Breast, ovary, neuroblastoma
	HRK	Pro-apoptotic BH3-only subfamily	Colon, stomach
DNA repair/ DNA damage response	hMLH1	Mismatch repair	Colon, stomach, endometrium
	MGMT	DNA alkylation repair	Colon, lung
	BRCA1	DNA damage response	Breast
Angiogenesis/ Hypoxia	THBS1	Angiogenesis inhibitor	Colon, neuroblastoma
	VHL	Ubiquitin ligase	Kidney
	BNIP3	Hypoxia-mediated apoptosis	Pancreas, colon, stomach
Chromatin regulation and transcription	HLTF	Helicase-like transcription factor	Colon
	RIZ1	Histone methyltransferase	Breast, stomach, colon
	HIC1	Transcriptional repressor	Breast
Signal transduction	RASSF1	RAS effector	Lung, colon, breast, ovary
	NORE1	RAS effector	Lung
	RUNX3	TGF-beta pathway	Stomach
	SOCS3	Inhibitor of JAK/STAT pathway	Lung
	PTPRO	Tyrosine phosphatase inhibitor	Lung
	SFRP	WNT signaling	Colon, stomach, breast
Hormone receptor	ER	Estrogen receptor	Colon, breast
	RAR-beta	Retinoic acid receptor	Colon, breast, leukemias
	PGR	Progesterone receptor	Breast
Cell attachment and invasion	CDH1	Cell adhesion	Breast, stomach, lung
	TSLC1	Cell adhesion	Lung, stomach,
	Claudin-7	Tight junction protein	Breast

5.1.4. Evidence supporting aberrant DNA methylation in causing cancer

- The genes involved are often tumor-suppressor genes
- The tissue distribution of methylation and mutations is often the same (when both events affect the same gene)
- There is an allelic exclusivity to the process, suggesting common selective advantage to methylation or mutation
- Aberrant DNA methylation occurs early, in aging and preneoplastic tissues, and therefore precedes neoplasia
- Reducing methylation prevents tumor formation in mice
- Reprogramming epigenetic patterns in cancer reverses the malignant phenotype

5.1.5. Research Issues in DNA methylation

Methylation and silencing – which comes first?

While there is ample evidence that methylation is enough to silence gene expression, there also arguments in favor of a silencing first event (transcriptional down-regulation), with methylation ‘locking in’ the silenced state. There is some evidence for this in the case of the E-cadherin gene. In many cases, however, no loss of transcriptional ability is found in methylated cells, arguing against this possibility. In one study, methylation of p16 in a colon cancer cell line was shown to be reversed by deletion of DNMT1 and DNMT3b. In these cells, p16 was reactivated early, but then became silenced with histone H3 lysine 9 methylation detectable in the promoter. The gene *subsequently* showed DNA methylation, arguing for the sequence silencing then methylation. Recently, a specific histone code indicating polycomb group protein binding has been associated with propensity to aberrant methylation in cancer.

Methylation and silencing – always associated?

While there are multiple examples of a good association between methylation and silencing, this is not always the case. Possible reasons for a lack of correlation between methylation and silencing include: (1) ‘Light’ methylation i.e. methylation of less than 10% of CpG sites in an island, which may not be enough to trigger silencing. (2) Methylation of CpG islands that are not in the promoters of genes. (3) Heterogeneous methylation, i.e. some cells in a given tumor are methylated but not others, leading to patchy silencing in the tumor. (4) Methylation of only one of several alternate promoters for a given gene.

Hypermethylation and aging

Increasing evidence suggests that some CpG islands become methylated in normal tissues as a function of age or time-dependent events. Whether this contributes to physiologic aging remains to be determined. In some cases, therefore, methylation is clearly a molecular clock and reflects the long life of neoplastic cells rather than selective advantage. Thus, some tissue-specific genes are methylated very commonly in cancers of tissues that do not express the genes in the first place. For example, MYOD1 is only expressed in muscle tissues. However, it is very frequently methylated in non-muscle cancers. There, one cannot argue that MYOD1 methylation causes cancer. Rather, evidence suggests that MYOD1 methylation increases linearly with the number of cell replication and functions as a molecular clock of the disease.

Hypermethylator phenotypes

The realization that some tumors have much higher levels of methylation than others do suggests a defined cause of methylation. Whether this relates to environmental exposures, mutations in the methylation machinery or other factors remains to be determined but this research may well shed significant light on the causes of hypermethylation in cancer.

Clinical applications

One of the most exciting aspects of this field is the possibility of intervening with DNA methylation inhibitors as treatment or prevention strategies in cancer. This is particularly intriguing because, as mentioned, hypermethylated genes usually have an intact coding sequence. In-vivo demethylation may therefore restore normal gene function in cancer cells and result in a non-toxic cancer treatment. There are at least two hypomethylating agents (5-azacytidine and 5-aza-2'-deoxycytidine) currently in clinical trials. Methylation has also shown promise as a screening tool for cancer as well as a prognostic factor in some tumors.

5.2. HDAC recruitment by fusion genes result in altered expression of target genes that contributes to tumor formation. For example, the PML/RAR translocation and the AML1/ETO translocation recruit HDACs to new target genes resulting in silencing.

5.3. Translocations involving Histone Acetyl Transferases: Several translocations in leukemia have been shown to fuse CBP/p300 to other proteins. Presumably, this results in aberrant histone acetylation and mis-expression of target genes.

5.4. Alterations in Histone Methylases: Translocations in the histone lysine 4 methyltransferase MLL (Trithorax) are common in leukemias, and presumably cause leukemia by down stream alterations of gene expression (e.g. HOX genes). Overexpression of the H3 Lysine 27 methyltransferase EZH2 is frequent in prostate and breast cancer, and is associated with a poor prognosis. Silencing of the RIZ1 histone methyltransferase is common in colon cancer.

5.5. Abnormalities of global chromatin regulators: Mutations in SNF5 cause rhabdoid tumors in children, and loss of HLTF is common in colon cancers. Both of these genes are global chromatin regulators, and cancer probably results from misexpression of target genes.

5.6. Imprinting defects:

Genetic imprinting is a process whereby only one allele of a gene is expressed, and the choice of expression is determined by the parent of origin of the alleles/chromosomes. For example, IGF2 is normally expressed from the paternal copy of chromosome 11, while the nearby gene H19 is expressed from the maternal copy. There are 100-200 imprinted genes in humans. In many (but not all) cases, imprinting is conserved between mice and humans. The function(s) of imprinting is not entirely clear, with the current leading theory being that imprinting evolved as a result of a male/female struggle for larger offspring (evolutionarily advantageous to males since these would have a higher chance of surviving and thus transmitting their genes) vs. smaller offspring (evolutionarily advantageous to females since, in situations of scarce resources, fetuses compete with mothers for nutrition etc. and smaller fetuses would increase the chances of females surviving pregnancy). In support of this, most imprinted genes play a role in development and diseases of imprinting result in faulty development. Also, many paternally expressed genes are growth factors, while maternally expressed genes suppress growth (although there are exceptions to this rule).

The molecular mechanisms of imprinting are still under investigation. It is clear that an imprint mark must be established in the gametes, but the nature of this mark is not entirely known. DNA methylation is essential to maintaining imprinting at many (but not all) imprinted loci, and it may play a role in imprint initiation as well, through methylation of DMRs (differentially methylated regions) in the gametes. Specific DNA methyltransferases (DNMT1/alternate splice and DNMT3L) are involved in establishing and/or maintaining imprinted patterns.

Altered imprinting can be found in developmental diseases (e.g. Beckwith- Wiedeman) and ‘Loss of Imprinting’ (bi-allelic expression) of the growth factor gene IGF2 has been implicated in Wilm’s tumor and recently in colon cancer. Thus, loss of imprinting could upregulate growth promoters and result in cancer. However, IGF2 imprinting may be more fluid than previously thought: It is not imprinted in normal liver or bone marrow, and stimulated lymphocytes go from monoallelic to biallelic expression. Thus, IGF2 LOI could also be a feature of proliferating cells and tissues, and may not necessarily be pathogenic in cancer.

6. Epigenetic Therapy

Rationale

Transcriptional defects are common in cancer (and other diseases). Epigenetically silenced genes typically have a normal nucleotide sequence and would encode for a normal functioning protein if silencing were reversed. Examples of this are shown below.

Silenced Gene	Function	Evidence of restoration of function by epigenetic therapy
ER	Estrogen Receptor	Restored expression from estrogen sensitive genes
RARB2	Retinoic acid receptor	Restored growth suppression by retinoic acid
MLH1	Mismatch repair	Correction of mismatch repair defect
P14/ARF	MDM2 inhibition	Restored normal cellular distribution of MDM2

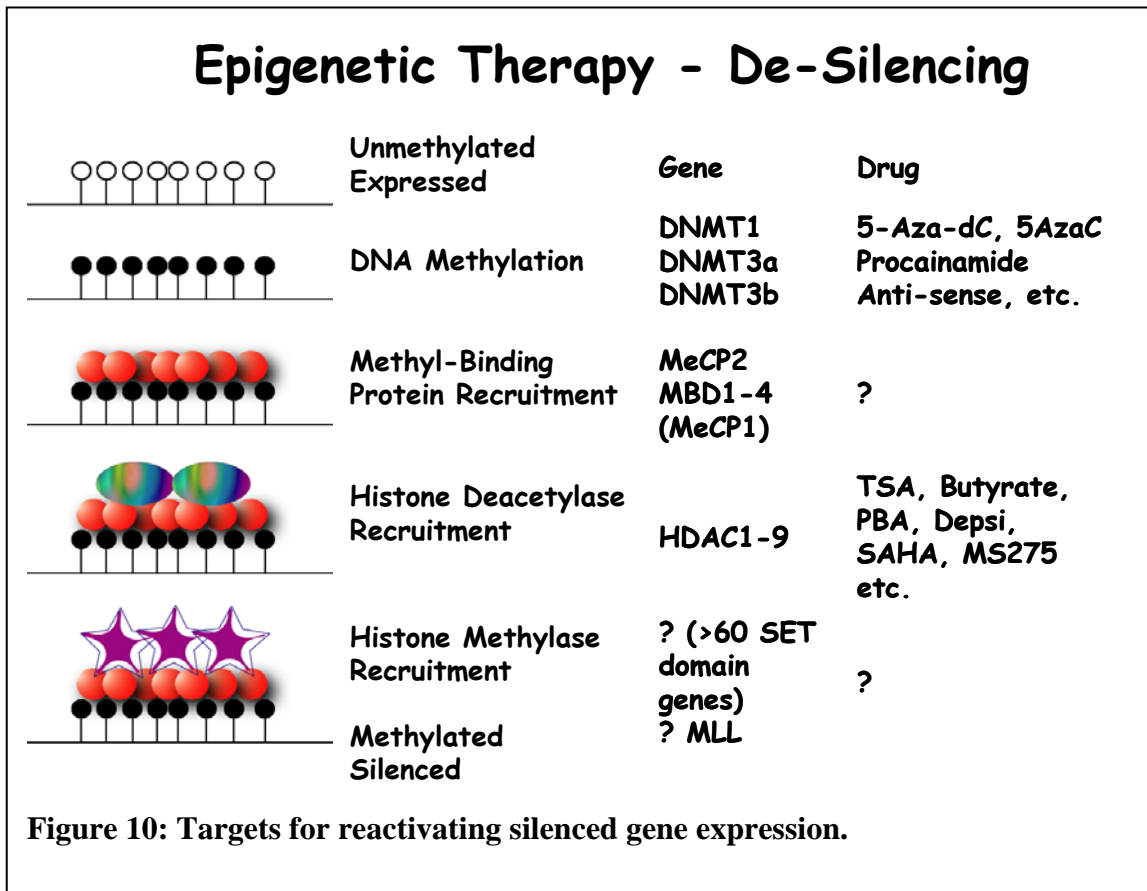
Thus, epigenetic therapy has the potential of ‘normalizing’ aberrant gene expression in cancer cells. This might be therapeutically advantageous in a number of ways. Cancer cells may now sense their own DNA damage and commit to senescence or apoptosis. Apoptotic threshold may be reduced by this therapy, such as when silenced apoptosis genes are reactivated. Cancer cells may also terminally differentiate. Cancer antigens may be unmasked by epigenetic therapy, which could result in enhanced immunity. Finally, gene reactivation may be exploited therapeutically. For example, restored RARB2 may make the cells more sensitive to ATRA, a retinoic acid. Restored expression of apoptosis genes may make the cells more sensitive to standard chemotherapeutic agents etc.

Targets

There are three approaches to potentially achieving epigenetic de-silencing (epigenetic therapy).

1. Indirect: If a specific molecular defect leads to silencing, then targeting this defect will lead to gene reactivation. For example, the PML/RAR fusion protein in APL is transcriptionally deregulated. ATRA promotes degradation of PML/RAR resulting in gene reactivation, differentiation and therapeutic benefit. Other fusion proteins in leukemias also result in transcriptional deregulation (e.g. MLL fusion proteins), and targeting these fusion proteins restores gene expression and could be of therapeutic benefit.
2. Direct transcriptional activation. Zinc finger proteins have been designed that can bind to specific promoters and activate gene expression. For cancer, this technology suffers from the same drawbacks as gene therapy, namely delivery to the cancer nucleus.

3. Targeting the silencing apparatus. Each step in silencing is mediated by known genes/proteins that either have known inhibitors or are candidates for drug development.



Based on the model shown above, therapeutic targeting of the silencing apparatus can include targeting DNA methylation, methyl-binding proteins, histone deacetylases and histone methylases. Currently, there are known inhibitors only of DNA methylation and histone deacetylases.

Take home points

- Epigenetic silencing is a ubiquitous if rarely used phenomenon in multicellular organisms.
- Silencing plays important roles in development/differentiation and genome defense.
- Silencing can be transcriptional, post-transcriptional (RNAi) or even mutational.
- Transcriptional silencing involves histone modifications in most organisms (particularly deacetylation and H3 lysine 9 dimethylation or H3 lysine K27 trimethylation) and DNA methylation in humans.
- DNA methylation and histone modifications form a stable silencing loop.
- Epigenetic silencing is frequently aberrant in cancer, with silencing of tumor-suppressor genes and activation/inactivation of genes by aberrant histone acetylation/deacetylation as common examples. Many of the genes affected fall in pathways critical to cancer development.
- Silencing can be reversed by DNA methylation inhibitors and/or histone deacetylase inhibitors. Other targets could also work (methyl-binding proteins, histone methyltransferases), but drugs that affect those have not yet been identified.

Additional reading

1-5

Reference List

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