

Epigenetic Changes

Chromatin regulation, gene expression and malignant behavior

DNA methylation

Histone regulation

Cancer phenotype

Dr Abu Suraih Sakhri

MBBS, MD (Pathology)

www.abusuraihsakhri.com

Teaching outcomes: Interpreting the cancer epigenome

- Distinguish DNA-sequence lesions from abnormal control of gene expression.
- Explain promoter methylation, histone marks, chromatin remodeling and enhancer contacts.
- Connect epigenetic lesions to nuclear appearance, tumor lineage, therapy and reversible resistance.

Molecular mechanism



Clinicopathologic meaning



Critical interpretation

Epigenetics regulates phenotype without changing the DNA code

- Epigenetic mechanisms change how genomic information is read, not the underlying base sequence.
- Their effects on transcription alter cell behavior, differentiation and disease phenotype.
- Cancer may have both sequence mutations and dysregulated epigenetic states.

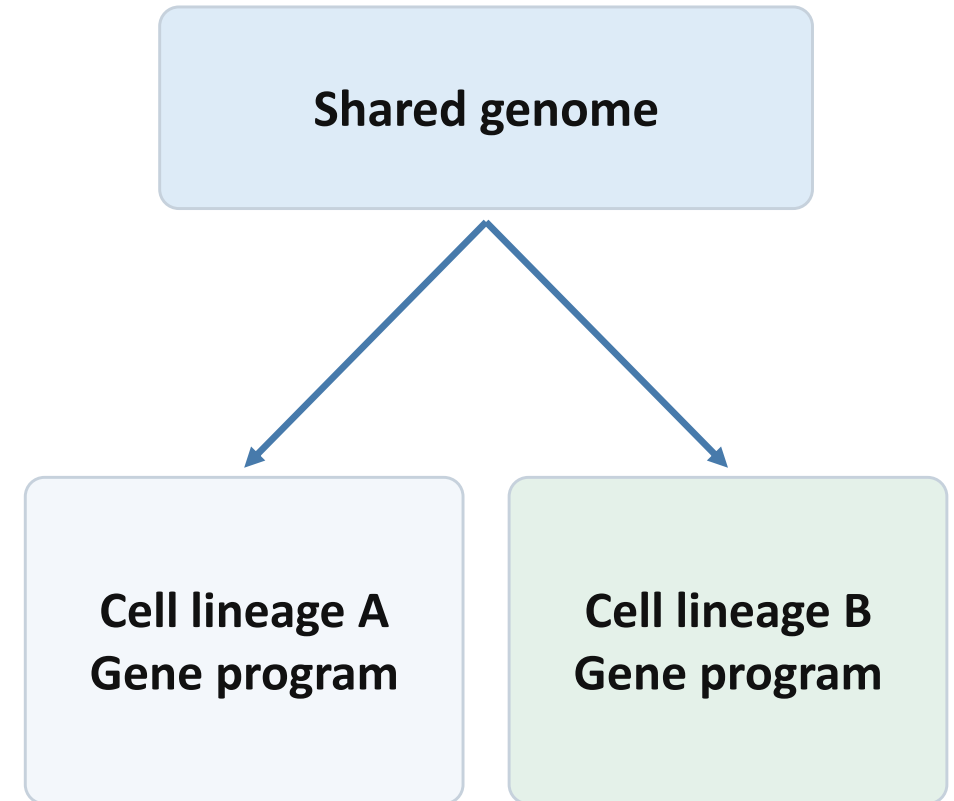
DNA sequence
Base order

Epigenetic control
Access and transcription

Distinct mechanisms • possible convergence

Identical genomes can support distinct cell identities

- Differentiated cell types express distinct gene programs despite nearly identical genomic DNA.
- Chromatin accessibility helps select which genes are active in each lineage.
- Cancer emerges within a pre-existing lineage-specific regulatory environment.



Four interacting controls shape transcription

- DNA methylation changes the chemical state of regulatory DNA.
- Histone modification changes chromatin packing and recruitment of regulatory proteins.
- Nucleosome positioning and long-range enhancer contacts control promoter access.

DNA methylation

Histone modifications

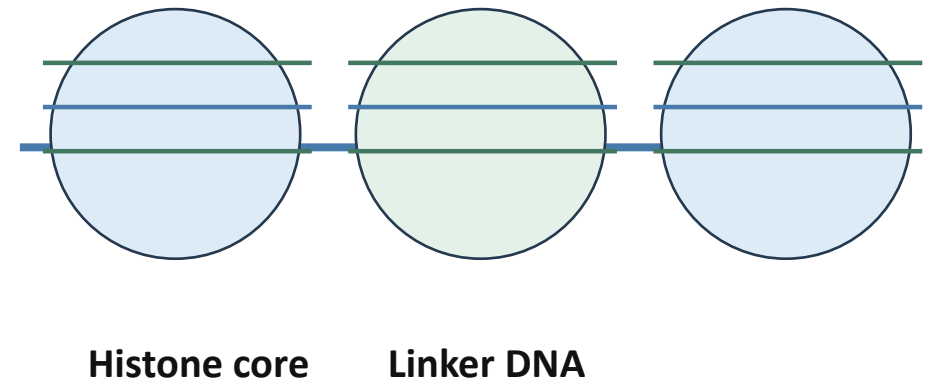
Nucleosome positioning

Enhancer–promoter looping

Chromatin packages DNA but also governs its use

- Nucleosomes are DNA wrapped around histone protein cores.
- Packing is not uniform: Chromatin can be compact or relatively accessible.
- Its organization controls whether transcriptional machinery reaches regulatory DNA.

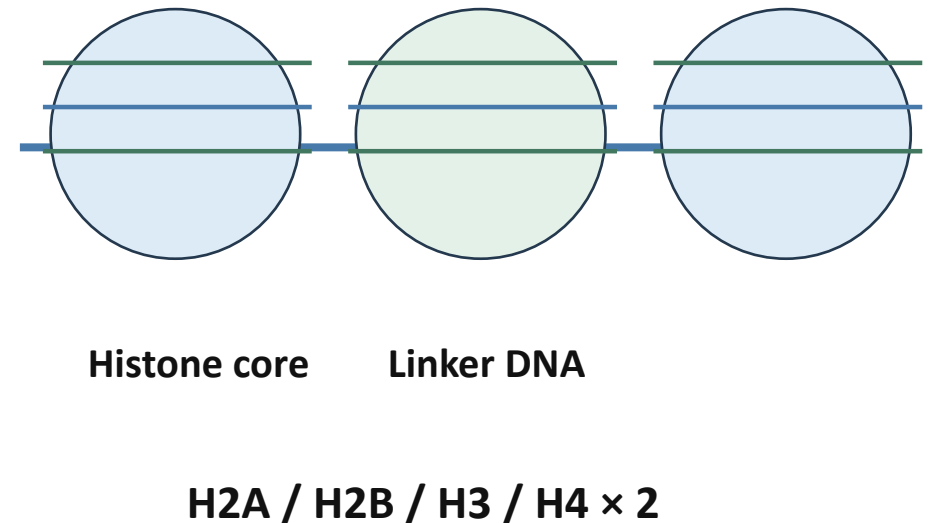
Original nucleosome schematic



The nucleosome is the fundamental regulatory unit

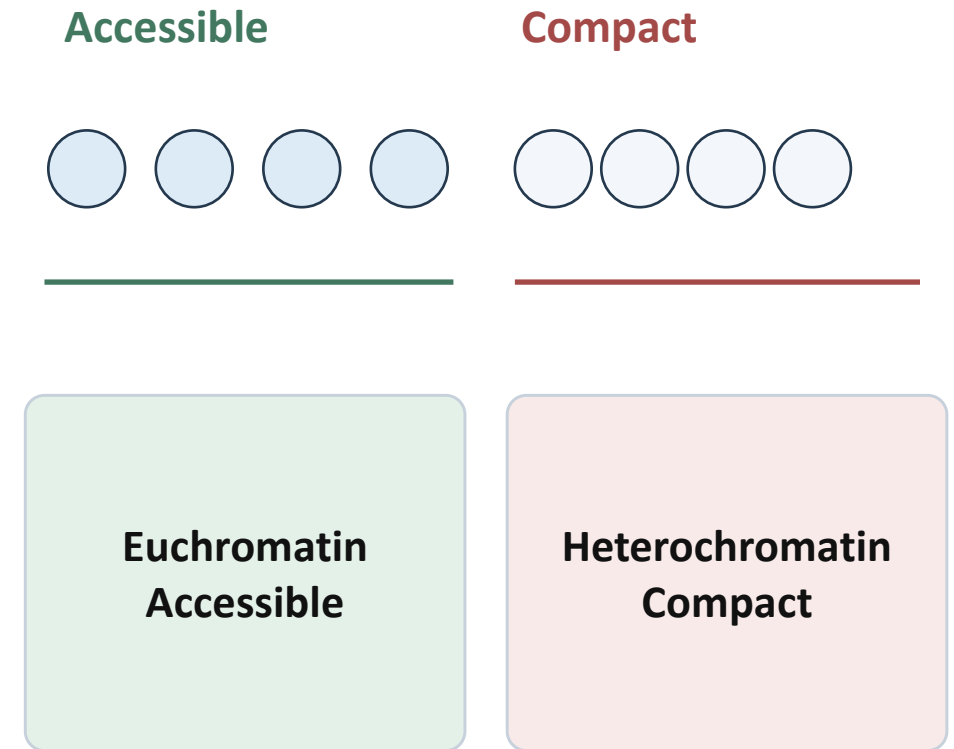
- The core octamer contains two copies each of histones H2A, H2B, H3 and H4.
- Approximately 147 DNA base pairs wrap around the histone core.
- Linker DNA connects nucleosomes; Histone H1 associates with linker regions.

Original nucleosome schematic



Euchromatin and heterochromatin represent different access states

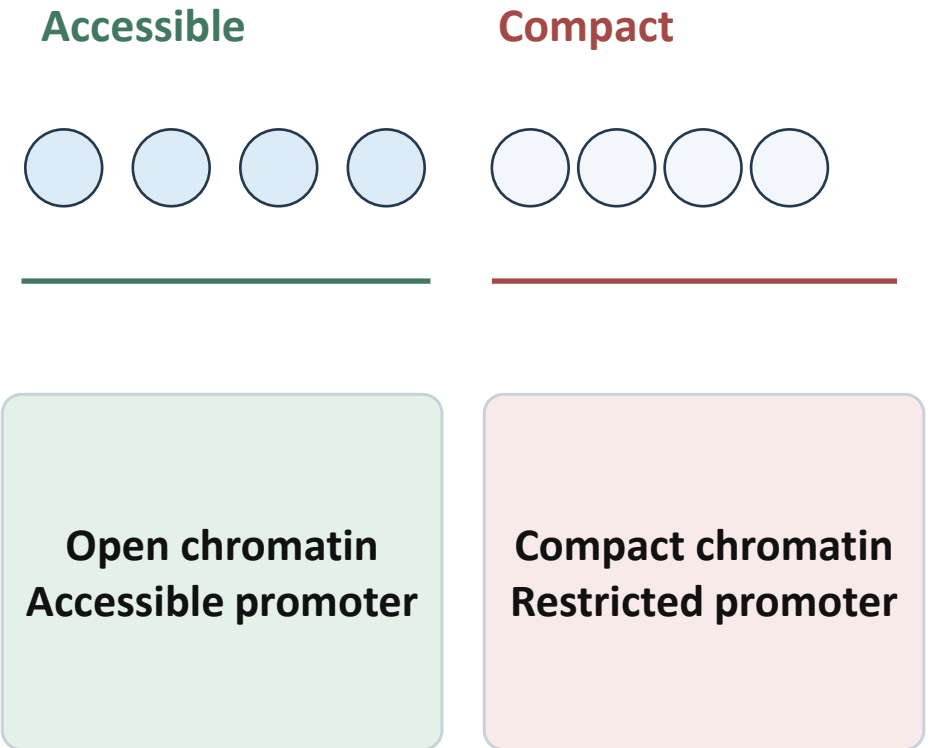
- Euchromatin is relatively dispersed and is associated with active transcription.
- Heterochromatin is cytochemically dense and generally transcriptionally inactive.
- These are functional chromatin states, not changes in DNA sequence.



Regulatory access determines transcription

Gene activity depends on access to promoter DNA

- Chromatin opening makes DNA more accessible to transcription factors and RNA polymerase.
- Chromatin compaction restricts access and generally reduces transcription.
- Local architecture—not merely the presence of a gene—determines whether it is expressed.



Regulatory access determines transcription

Writers, readers and erasers interpret histone marks

- Writers deposit chemical modifications on histone proteins.
- Readers recognize specific marked histones and recruit regulatory activities.
- Erasers remove marks; Remodelers reposition nucleosomes on DNA.

Writer

Deposits a mark

Reader

Binds a mark

Eraser

Removes a mark

Remodeler
Repositions
nucleosomes

DNA marks and histone marks act at different substrates

- DNA methyltransferases chemically modify cytosine residues in DNA.
- Histone-modifying enzymes act on protein residues in nucleosomes.
- The two systems cooperate to create context-specific transcriptional states.

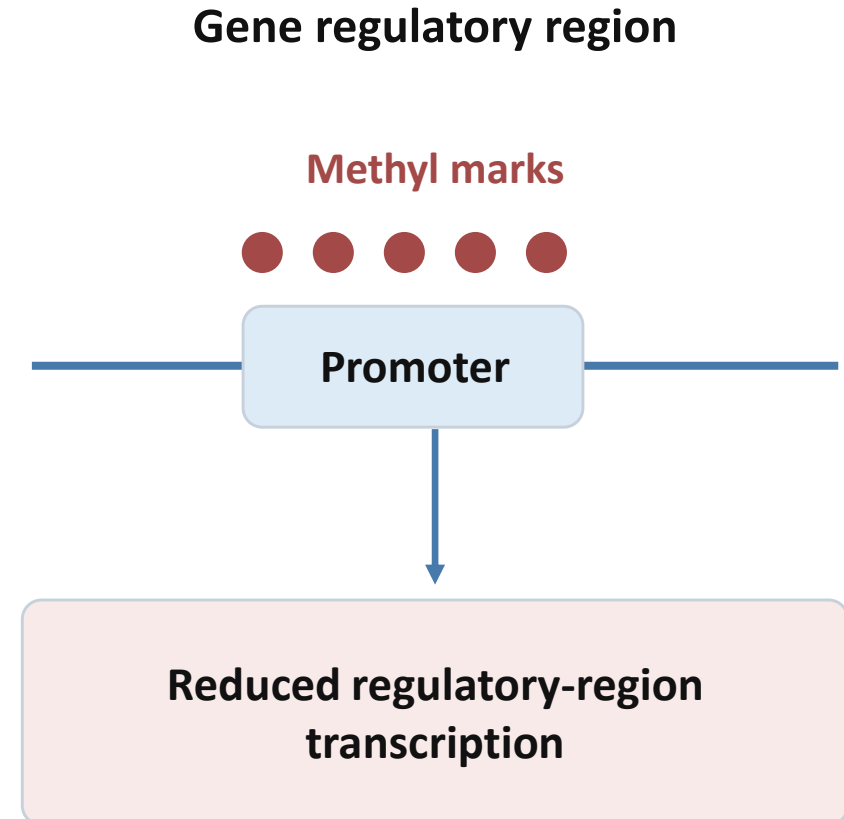
**DNA
Cytosine
methylation**

**Histones
Protein
modifications**

Distinct mechanisms • possible convergence

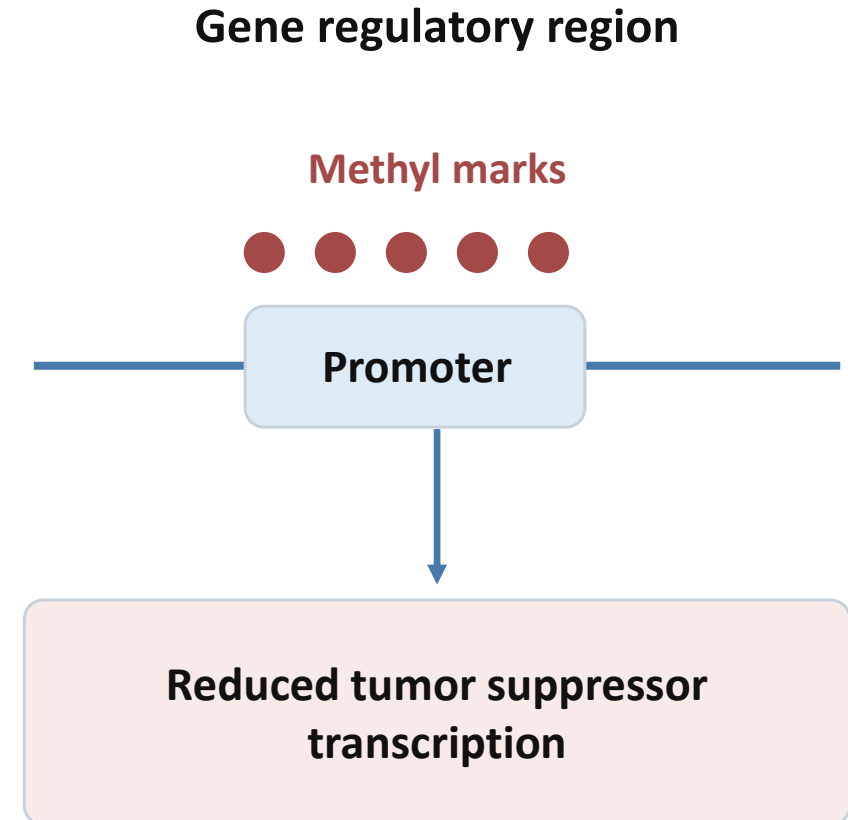
DNA methylation can silence regulatory regions

- Methylation of DNA is generated by DNA methyltransferases.
- High methylation at gene regulatory elements generally associates with transcriptional silencing.
- The meaning of methylation must be interpreted in its genomic location.



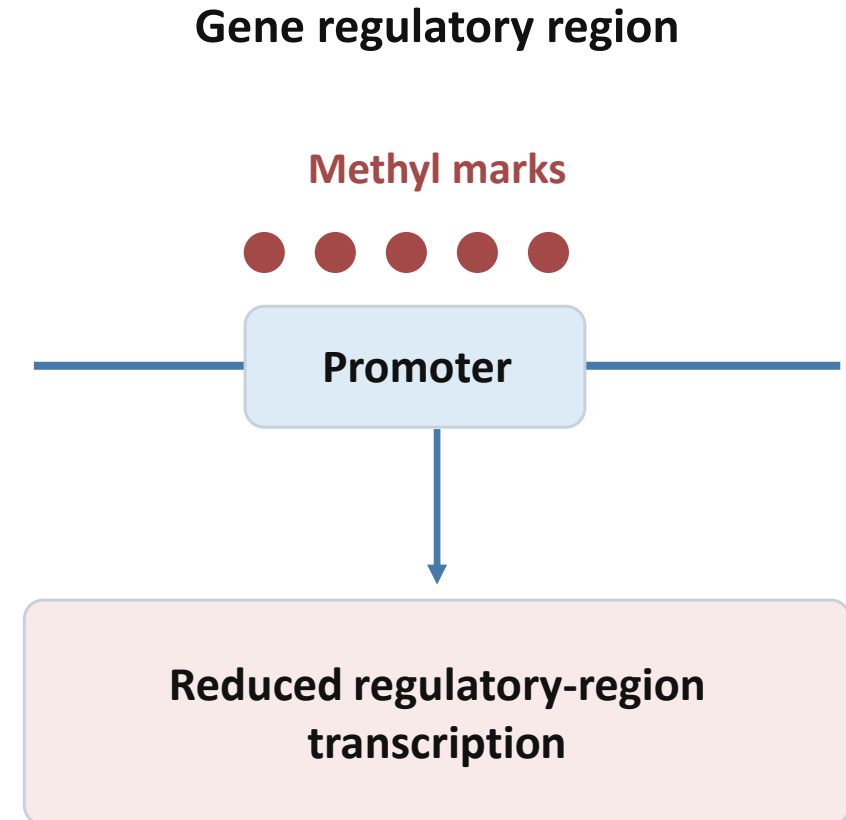
Promoter methylation can switch off a tumor suppressor

- Selective hypermethylation at a tumor-suppressor promoter can diminish its transcription.
- Protein output is reduced even when that allele retains an intact coding sequence.
- This is an epigenetic route to loss of tumor suppressor activity.



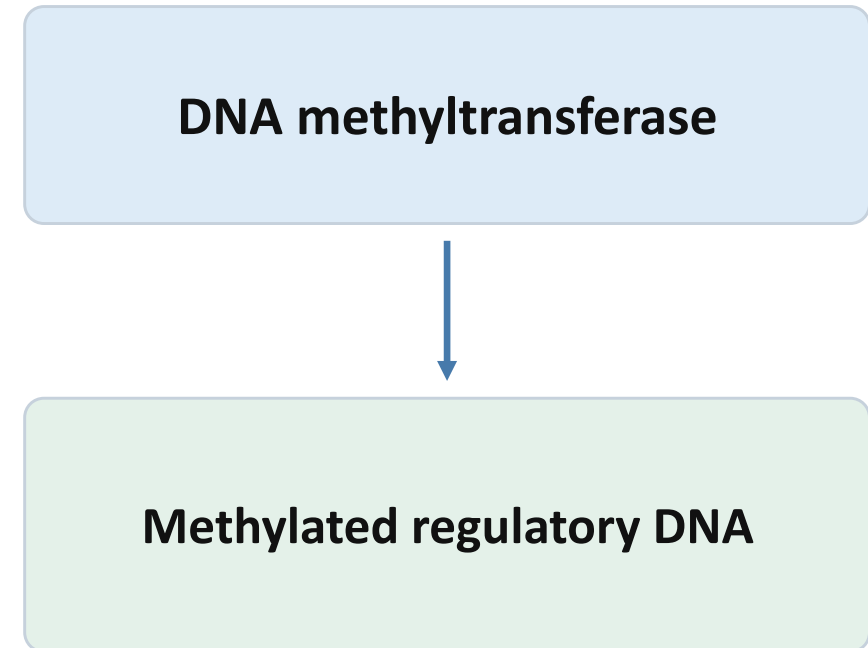
CpG dinucleotides provide DNA methylation sites

- The chemical modification targets cytosine bases, commonly at CpG dinucleotides.
- A promoter region can acquire abnormal local hypermethylation in cancer.
- The regulatory consequence is gene-dependent; Methylation is not a universal on–off label.



DNA methyltransferases write a reversible epigenetic mark

- DNA methyltransferase enzymes establish DNA methylation states.
- The cell also possesses processes and proteins that reverse or interpret methylation.
- Cancer-associated mutations can affect proteins controlling the methylation landscape.

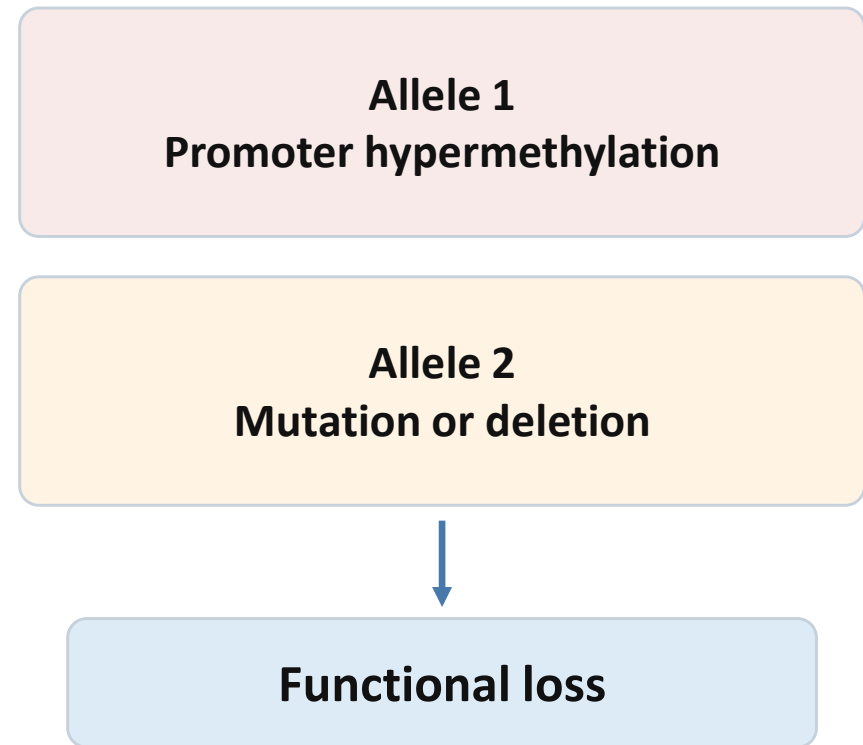


Enzymatically mediated change

Tumor suppressor silencing may involve both alleles

- Promoter hypermethylation affecting one tumor-suppressor allele.
- The partner allele may be lost through deletion or disabled by mutation.
- Combined loss can eliminate tumor-suppressive function without identical lesions in both copies.

Two copies of a tumor suppressor



Promoter hypermethylation is distinct from coding mutation

- Coding mutation alters the DNA base sequence of the gene.
- Promoter hypermethylation changes transcriptional regulation at an otherwise potentially intact allele.
- Both can converge on reduced functional tumor-suppressor protein.

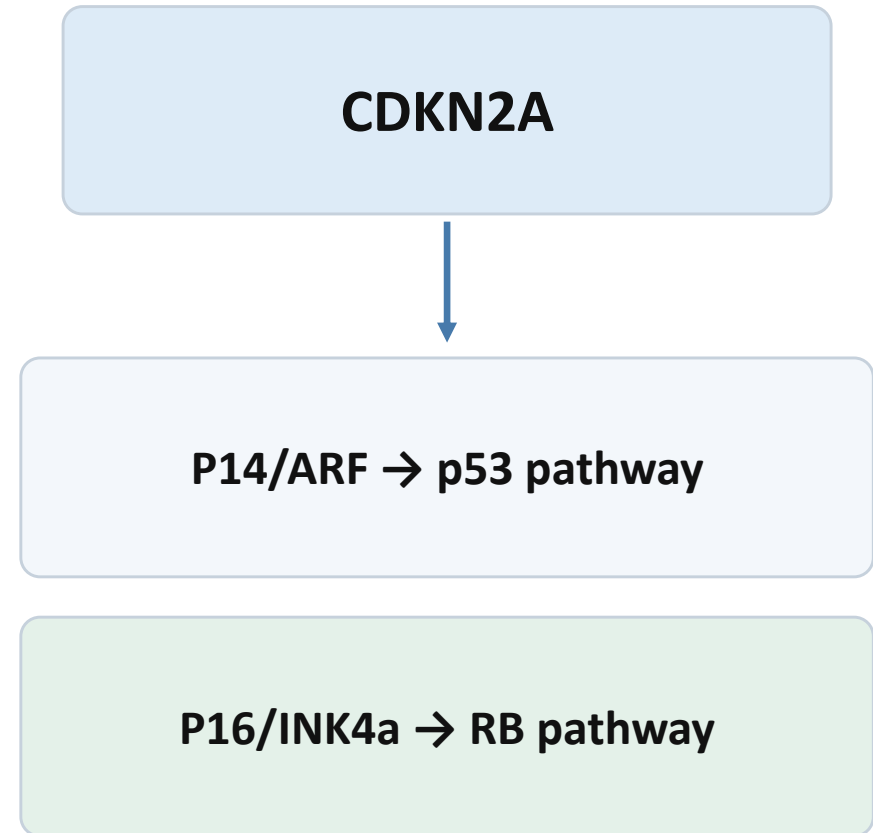
Coding mutation
Altered gene
product

Promoter
methylation
Reduced
transcription

Distinct mechanisms • possible convergence

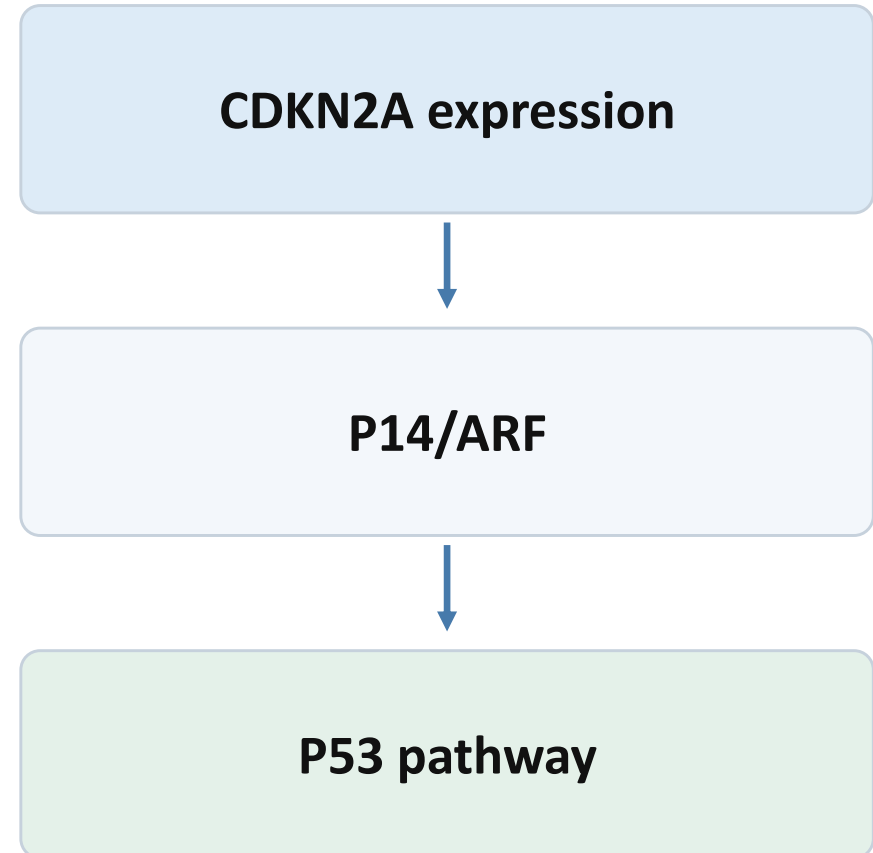
CDKN2A illustrates epigenetic tumor suppressor loss

- The CDKN2A locus can be hypermethylated in several malignancies.
- This complex locus encodes the p14/ARF and p16/INK4a tumor suppressors.
- These proteins feed into the p53 and RB tumor-suppressive pathways, respectively.



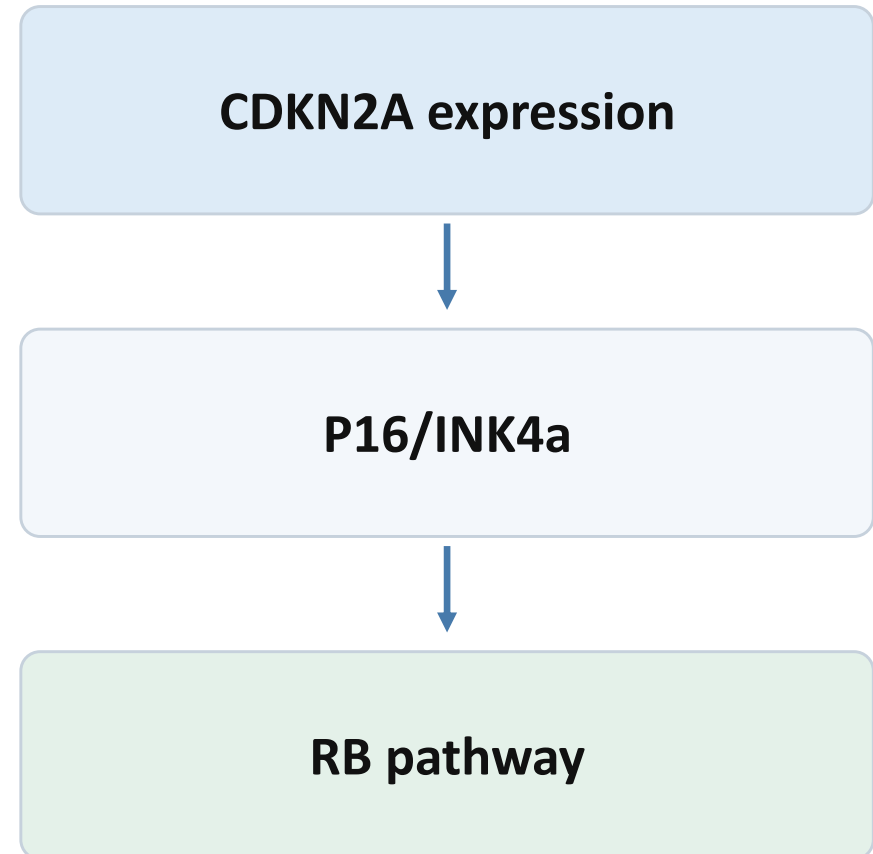
P14/ARF supports the p53 defense program

- One CDKN2A-derived tumor suppressor is p14/ARF.
- Its activity to enhancement of the p53 tumor-suppressor pathway.
- Epigenetic silencing of the locus can therefore affect a major anticancer defense.



P16/INK4a contributes to RB-mediated growth control

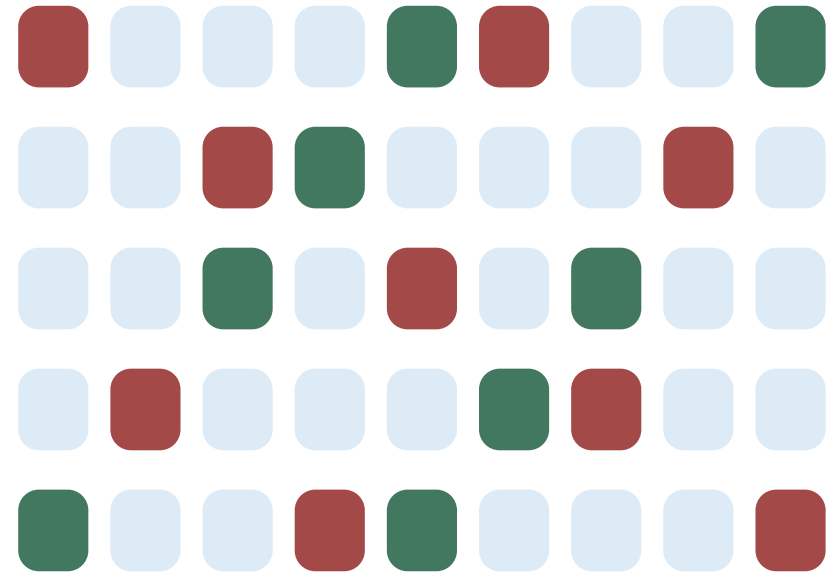
- A second CDKN2A-derived protein is p16/INK4a.
- Its tumor-suppressive effect enhances RB pathway activity.
- Loss of CDKN2A expression can therefore compromise RB-associated growth restraint.



Global methylation disturbance affects multiple genes

- Many cancers have abnormal DNA methylation extending beyond a single promoter.
- Some regions may gain methylation while others lose it.
- Consequences include inappropriate increases or decreases in expression across many loci.

Schematic methylation map (not quantitative)



Abnormal gain or loss across multiple regions

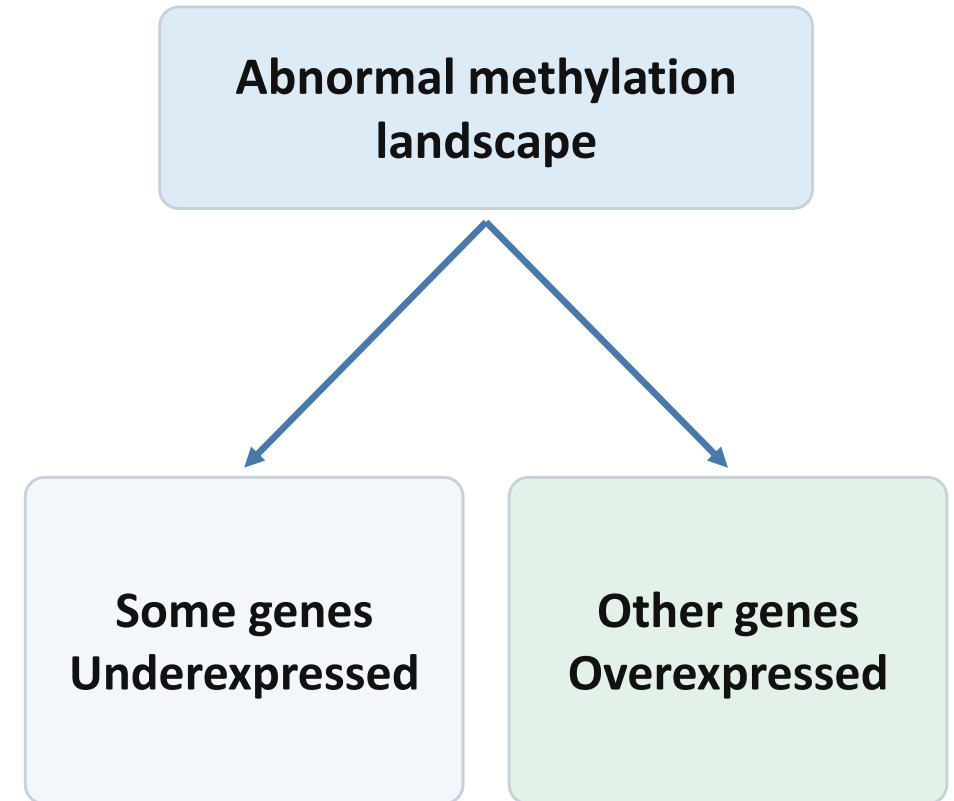
Local and genome-wide methylation changes are not synonyms

- Local promoter hypermethylation offers a mechanism for suppressing a specific gene.
- Global dysregulation describes an altered genome-wide pattern of methylation.
- Both may coexist in an individual cancer cell.

Feature	Local change	Genome-wide change
Scale	A promoter / locus	Many genomic regions
Effect	Silencing of a target gene	Diverse expression changes
Cancer link	Tumor-suppressor inactivation	Regulatory network disruption

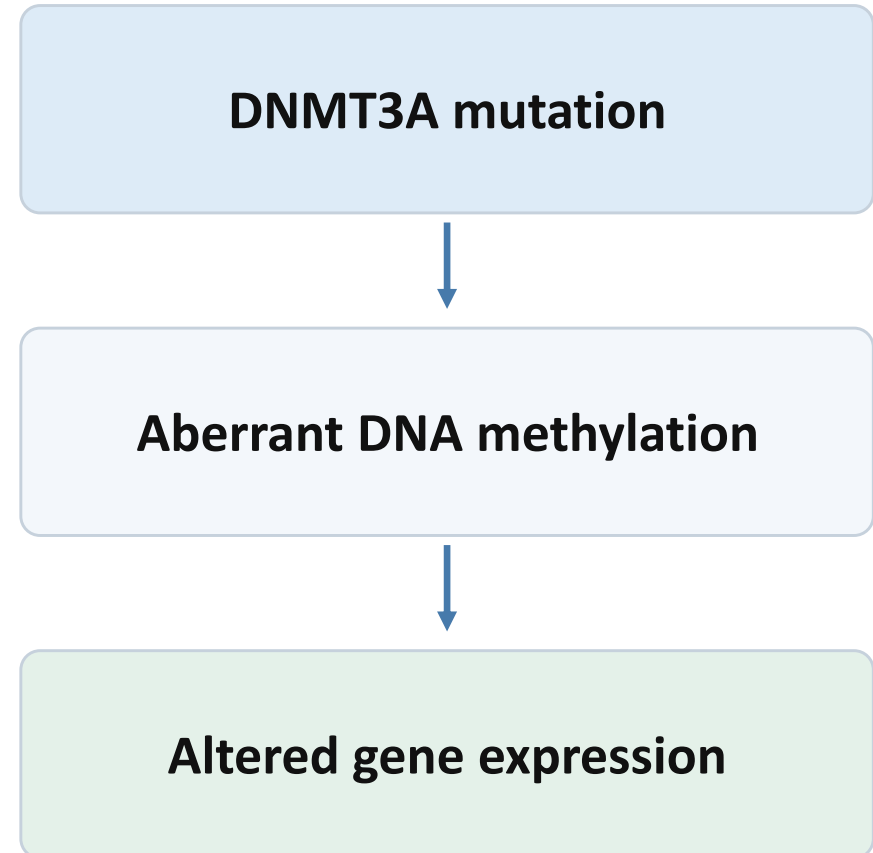
The expression effect of altered methylation is context-specific

- Genome-wide changes do not uniformly increase or decrease all transcripts.
- A gene may be overexpressed or underexpressed according to local regulatory changes.
- Methylation findings require locus-level interpretation rather than a single global direction.



Acute myeloid leukemia provides a methylation-regulator example

- DNMT3A among DNA-methylation regulators mutated in acute myeloid leukemia.
- A mutation in an epigenetic enzyme can disturb expression of numerous downstream genes.
- This links a genetic driver lesion to an abnormal epigenomic state.



Genetic lesions can reprogram epigenetic state

- Mutations in genes encoding epigenetic regulators are recurrent in cancers.
- The resulting defective proteins can alter methylation, histone marks or nucleosome positioning.
- Genomic alteration and epigenomic dysregulation are therefore mechanistically connected.

Regulator gene mutation



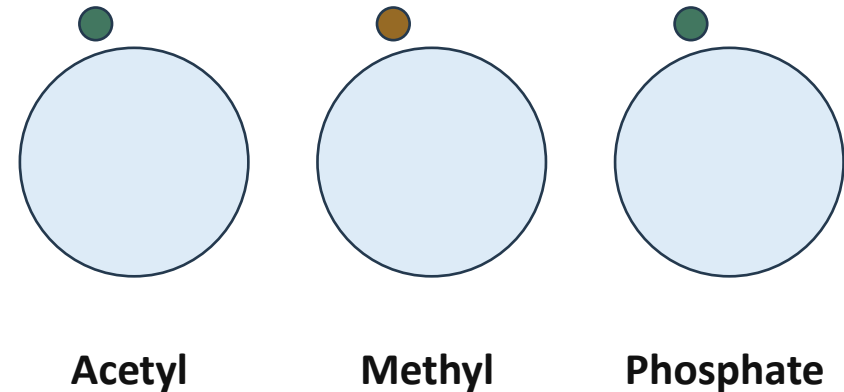
Epigenomic disruption



Malignant gene program

Histone modifications change transcriptional competence

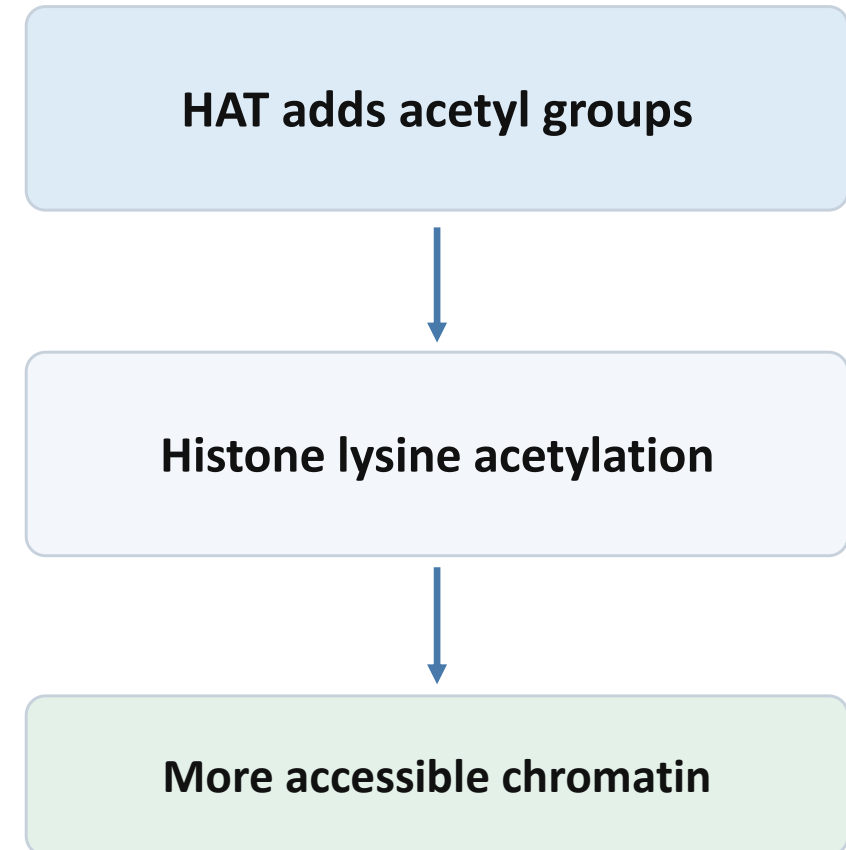
- Histone proteins undergo acetylation, methylation and phosphorylation.
- The effect depends on the modification and the specific residue affected.
- Histone marks can recruit factors that open or compact nearby chromatin.



Effect depends on mark and position

Histone acetylation generally favors accessible chromatin

- Histone acetyltransferases add acetyl groups to lysine residues.
- The modification generally promotes more open chromatin and gene transcription.
- This is a tendency, not a rule that every acetylation event independently activates every gene.



Histone acetyltransferases act as chromatin writers

- Histone acetyltransferases (HATs) catalyze addition of acetyl marks.
- Open chromatin makes regulatory DNA more accessible to transcriptional machinery.
- Alteration of acetylation regulators in cancer can reshape the transcriptional program.

Histone acetyltransferase



Acetylated histone lysines

Enzymatically mediated change

Histone deacetylases remove the acetyl mark

- Histone deacetylases (HDACs) erase acetyl groups from histones.
- Their activity generally favors chromatin condensation and reduced transcription.
- Because the mark is enzymatically removable, this system can be pharmacologically targeted.

Histone deacetylase

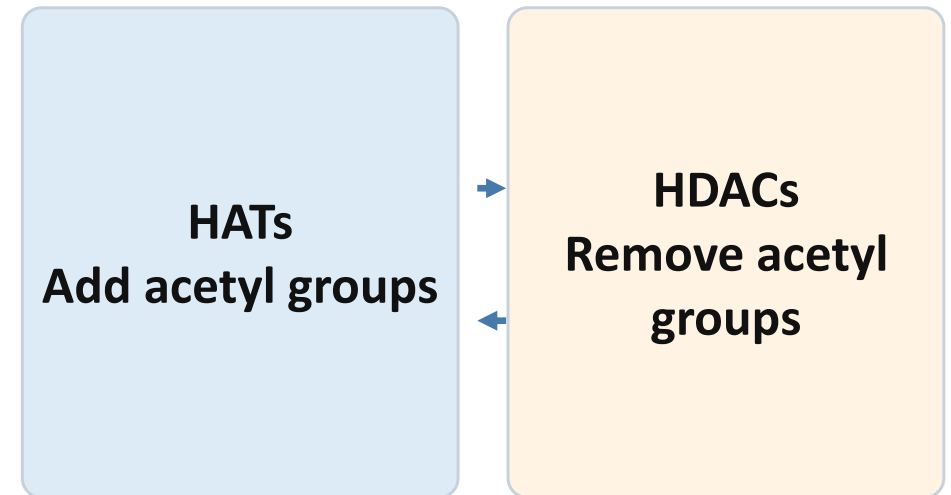


Deacetylated histone lysines

Enzymatically mediated change

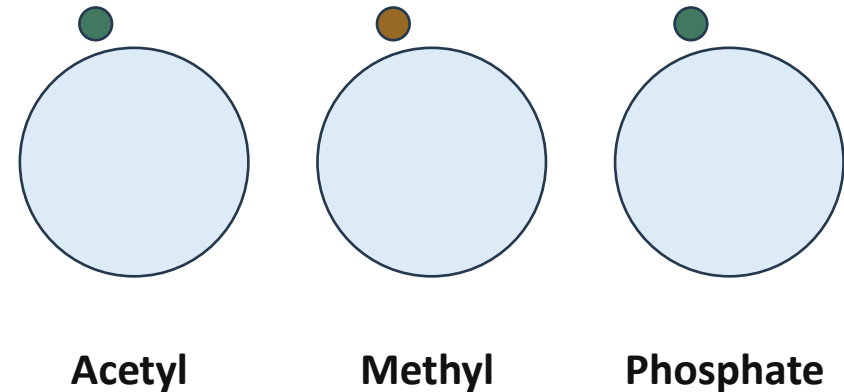
Histone acetylation is a dynamic balance

- Acetyltransferases and deacetylases have opposing enzymatic activities.
- The transcriptional state can shift as marks are written or erased.
- Net expression changes depend on the broader chromatin regulatory context.



Histone methylation is not uniformly repressive

- Specific enzymes methylate lysine and arginine residues on histones.
- Methylation of distinct histone lysines can associate with activation or repression.
- The position of the mark is crucial to its functional meaning.



Effect depends on mark and position

Residue identity determines the meaning of a histone mark

- Two methylated histones need not have equivalent transcriptional effects.
- Readers recognize particular modification patterns and recruit appropriate regulators.
- Interpreting a mark requires its biochemical and chromosomal context.

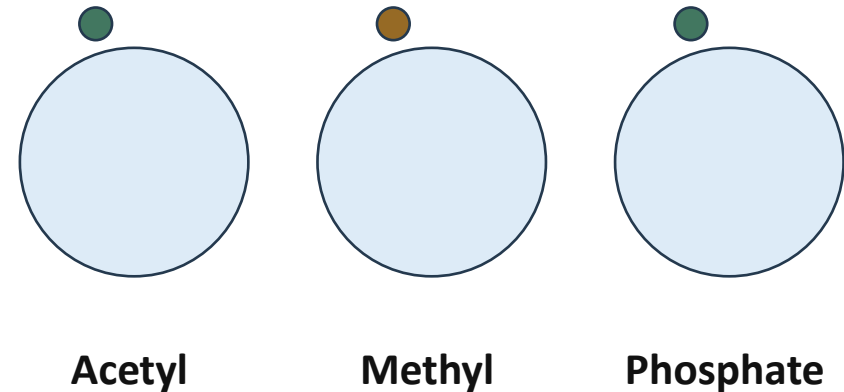
Methyl mark A
Activation-
associated

Methyl mark B
Repression-
associated

Distinct mechanisms • possible convergence

Histone phosphorylation can alter access in either direction

- Histone serine residues can acquire phosphate groups.
- Depending on the affected position, associated chromatin may open or compact.
- The mark cannot be interpreted correctly without its specific molecular context.



Effect depends on mark and position

Readers translate marks into cellular consequences

- Chromatin reader proteins bind modified histones bearing recognized marks.
- They help recruit or organize downstream regulatory activities.
- A mark influences expression through the proteins and complexes that respond to it.

Writer

Deposits a mark

Reader

Binds a mark

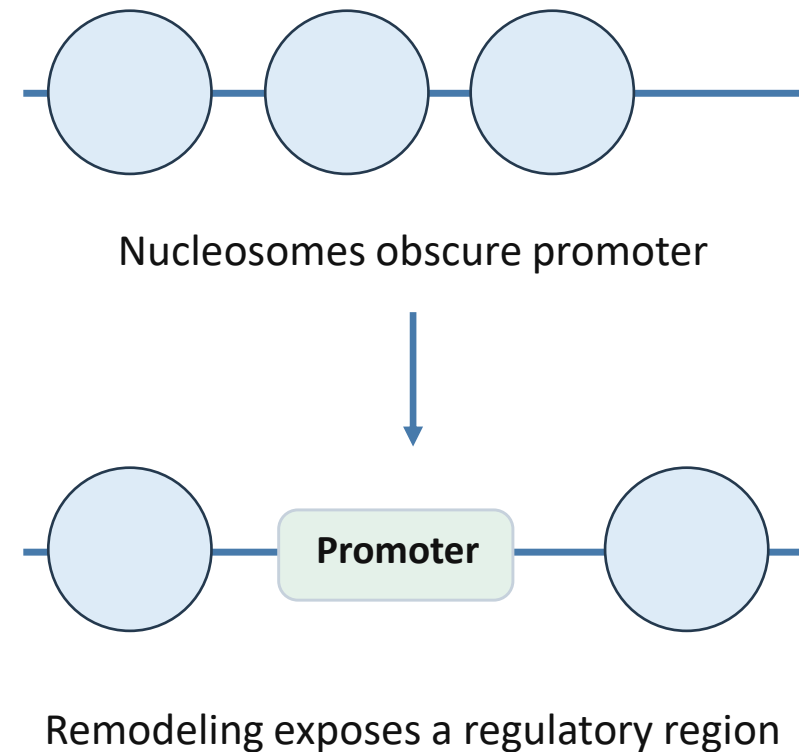
Eraser

Removes a mark

Remodeler
Repositions
nucleosomes

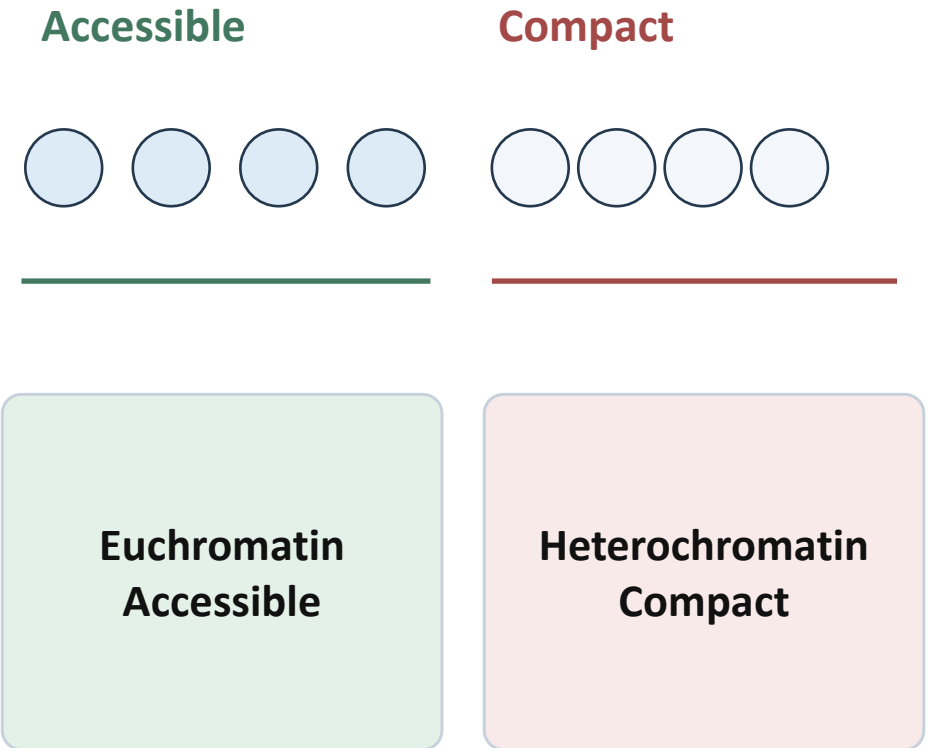
Chromatin remodelers move nucleosomes on DNA

- Remodeling complexes reposition nucleosomes relative to regulatory DNA.
- This can expose or conceal promoters and other regulatory sites.
- Cancer-associated lesions in remodeling components can alter access to entire gene programs.



Nucleosome positioning is independent of gene sequence

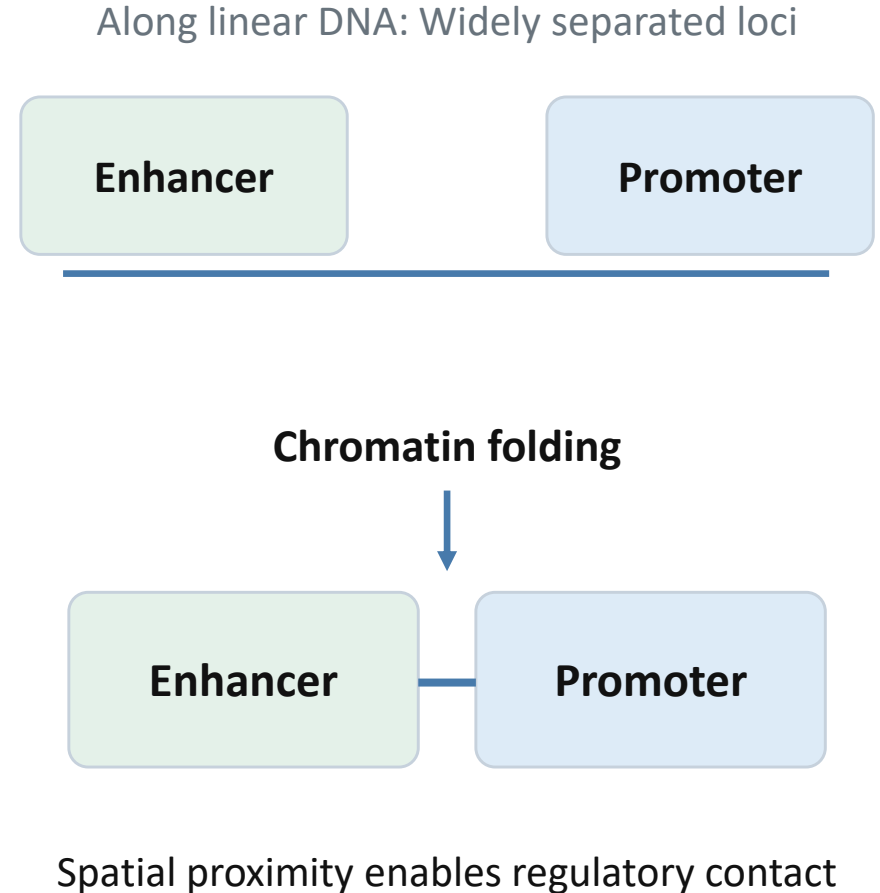
- The same promoter sequence can be accessible in one chromatin configuration.
- A differently placed nucleosome can obscure that regulatory sequence.
- Distinct gene activity does not require alteration of the underlying promoter DNA.



Regulatory access determines transcription

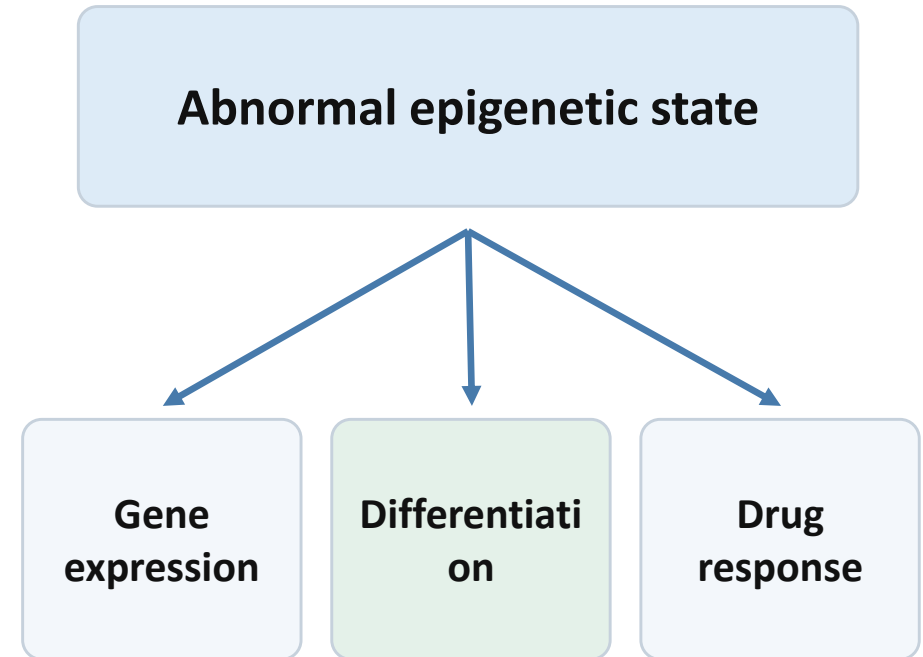
Enhancer–promoter looping adds a three-dimensional layer

- Chromatin organizing factors modulate long-distance contacts along DNA.
- An enhancer may be brought near a promoter by higher-order looping.
- Altered genome organization can influence the strength of transcriptional programs.



The malignant epigenome reflects several interacting layers

- Promoter methylation, histone marks and nucleosome placement jointly influence access.
- Enhancer contacts add spatial control over transcription.
- A mutation in one regulatory component can propagate to many target genes.

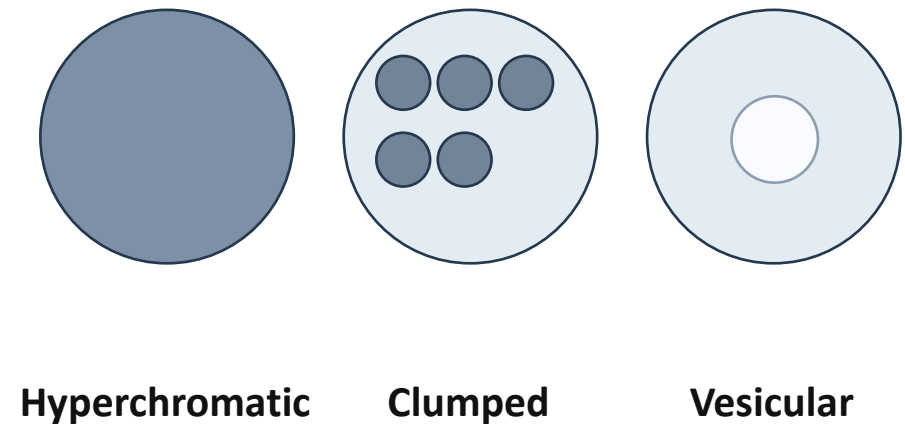


Schematic: Interacting downstream outcomes

Abnormal nuclear appearance reflects chromatin disturbance

- Cancer nuclei may show hyperchromasia, chromatin clumping or clearing.
- These histomorphologic alterations to disordered chromatin organization.
- Nuclear morphology therefore provides a visible correlate, not a complete readout, of epigenomic regulation.

Schematic nuclear chromatin appearances

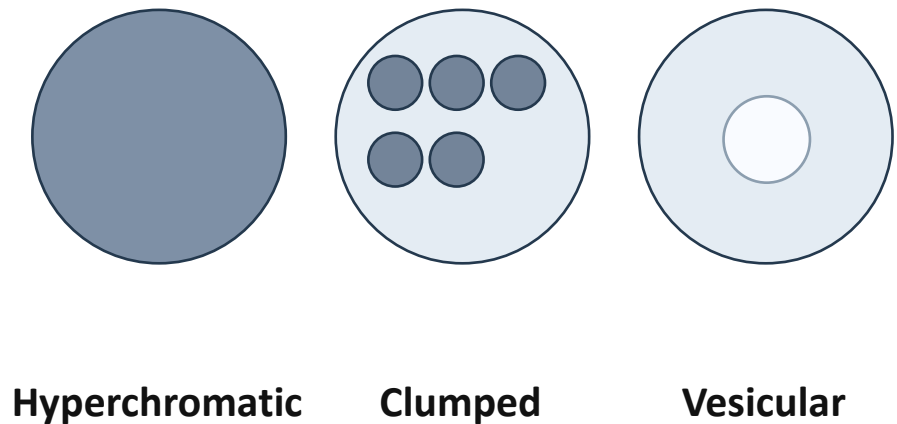


Concept diagram; Not a histology specimen

Hyperchromasia is a morphologic observation—not a methylation assay

- Hyperchromatic tumor nuclei stain more darkly at light microscopy.
- Chromatin organization contributes to this appearance.
- The finding does not identify which epigenetic enzyme or promoter is abnormal.

Schematic nuclear chromatin appearances

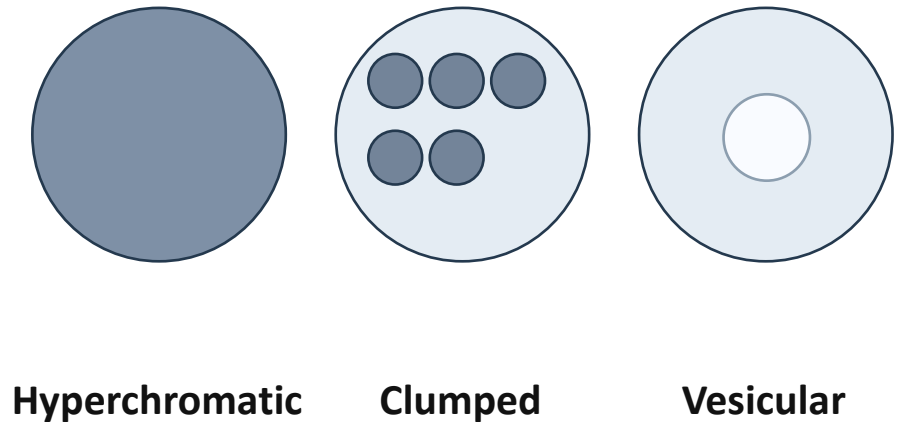


Concept diagram; Not a histology specimen

Chromatin clumping and clearing are alternative patterns

- Clumped chromatin yields coarse, uneven nuclear density.
- Vesicular chromatin describes a paler, more open-appearing nuclear pattern.
- Both may be encountered in malignancy; Neither specifies a unique molecular lesion.

Schematic nuclear chromatin appearances



Concept diagram; Not a histology specimen

Microscopic appearance cannot specify the epigenetic genotype

- Chromatin texture may be abnormal in a cancer biopsy.
- Determining whether a writer, eraser or remodeler is mutated requires molecular testing.
- The slide-level distinction prevents overinterpretation of histology.

**Morphology
Chromatin
appearance**

**Molecular
analysis
Specific gene
lesion**

Distinct mechanisms • possible convergence

Cancer genomes repeatedly disrupt epigenetic regulators

- Sequencing has revealed recurrent mutations in genes for DNA and histone regulation.
- Genes encoding nucleosome-positioning machinery are also affected.
- Some driver mutations directly involve genes encoding histone proteins.

DNA methylation genes

Histone-modifier genes

Chromatin-remodeler genes

Histone protein genes

Regulatory gene lesions cluster by biochemical function

- Methylation regulators change DNA or histone methyl marks.
- Acetylation enzymes influence histone acetyl marks.
- Remodelers affect nucleosome placement; Histone-gene mutations alter nucleosome components.

DNA methylation DNMT3A

Histone methylation KMT2A; KMT2D

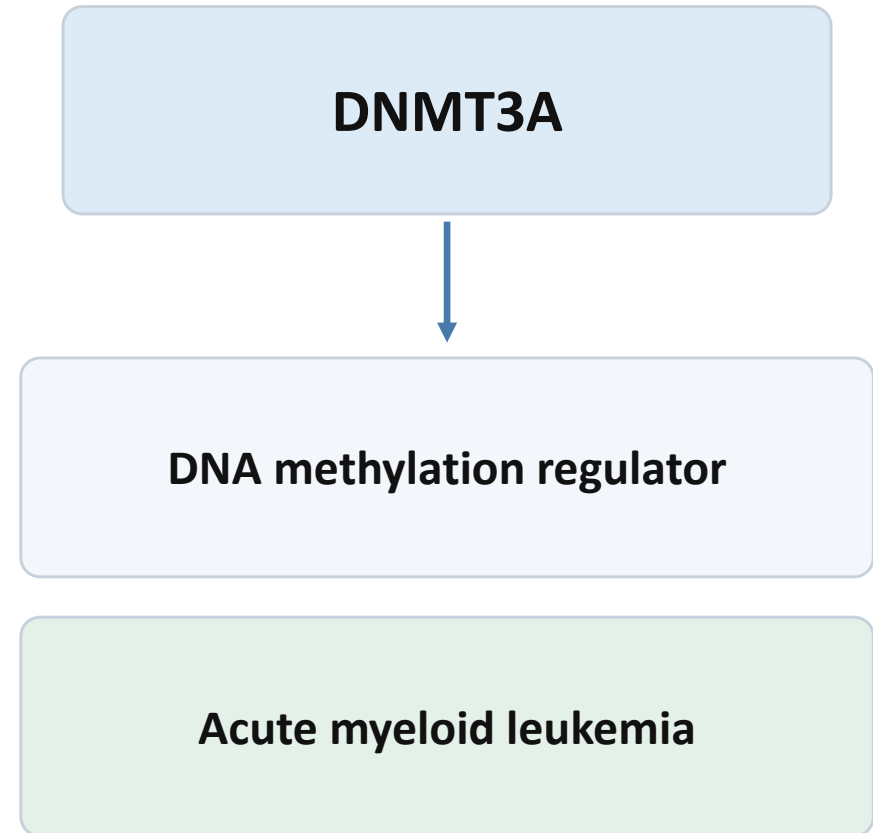
Histone acetylation CREBBP; EP300

Chromatin remodeling ARID1A; SNF5; PBRM1

Histone components H3F3A; HIST1H3B

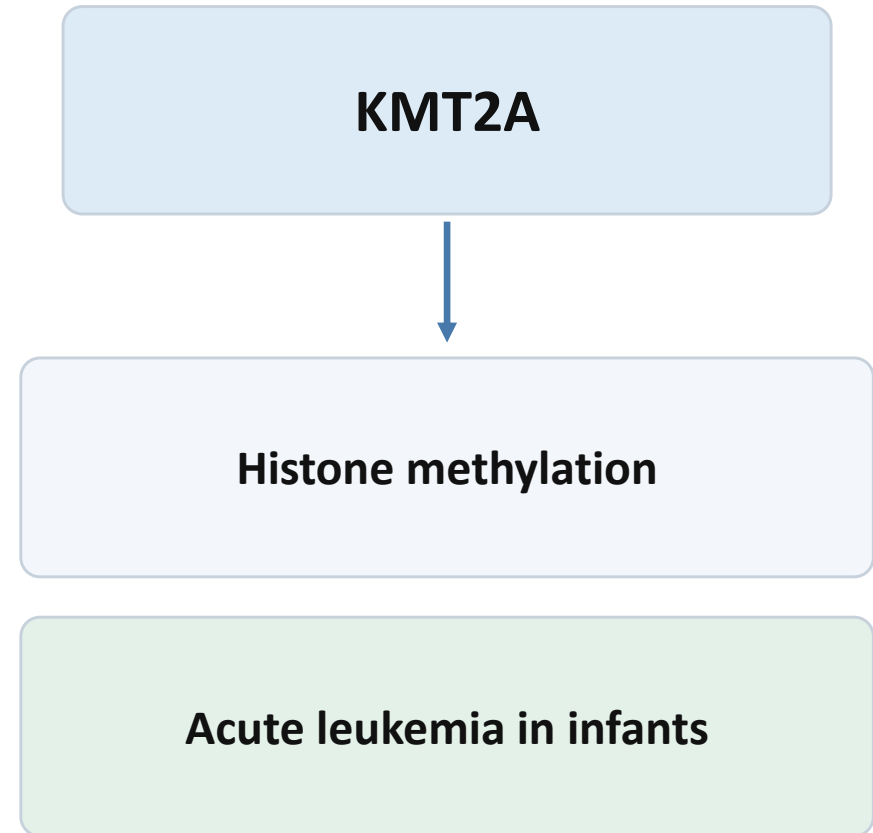
DNMT3A mutations connect DNA methylation to leukemia

- DNMT3A is listed as a DNA methylation regulator Table 7.9.
- Acute myeloid leukemia is an example of a cancer harboring DNMT3A mutations.
- The target is the regulatory machinery, not merely a downstream gene promoter.



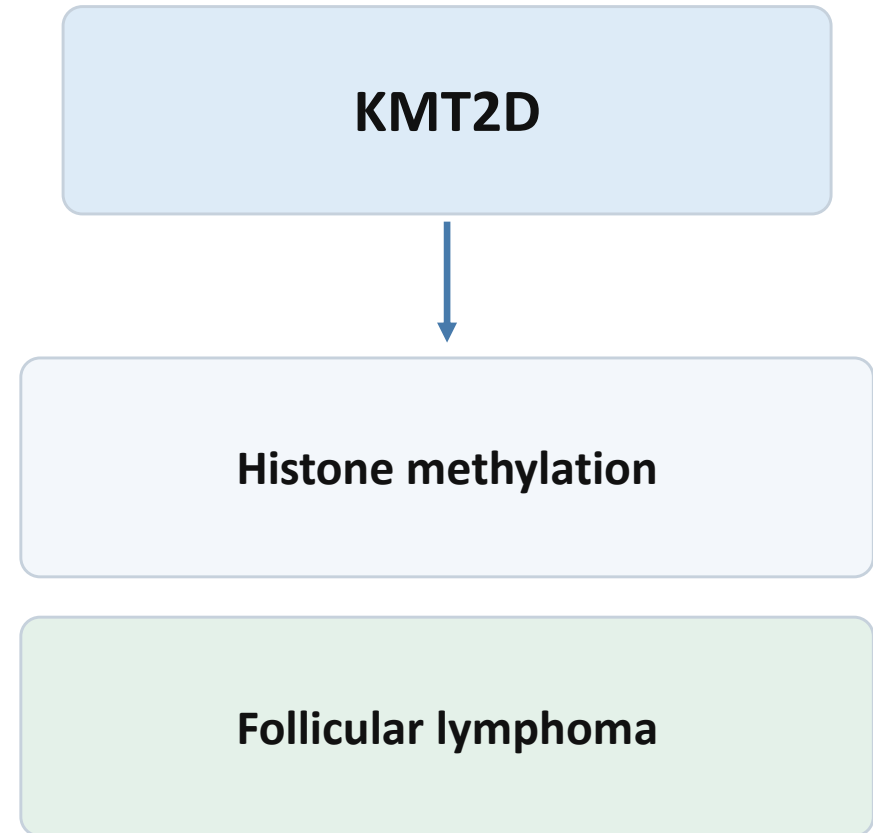
KMT2A highlights histone methylation in infant leukemia

- KMT2A is listed among genes with a histone-methylation function.
- Acute leukemia in infants as an associated tumor category.
- The example illustrates the link between chromatin regulation and malignant hematopoiesis.



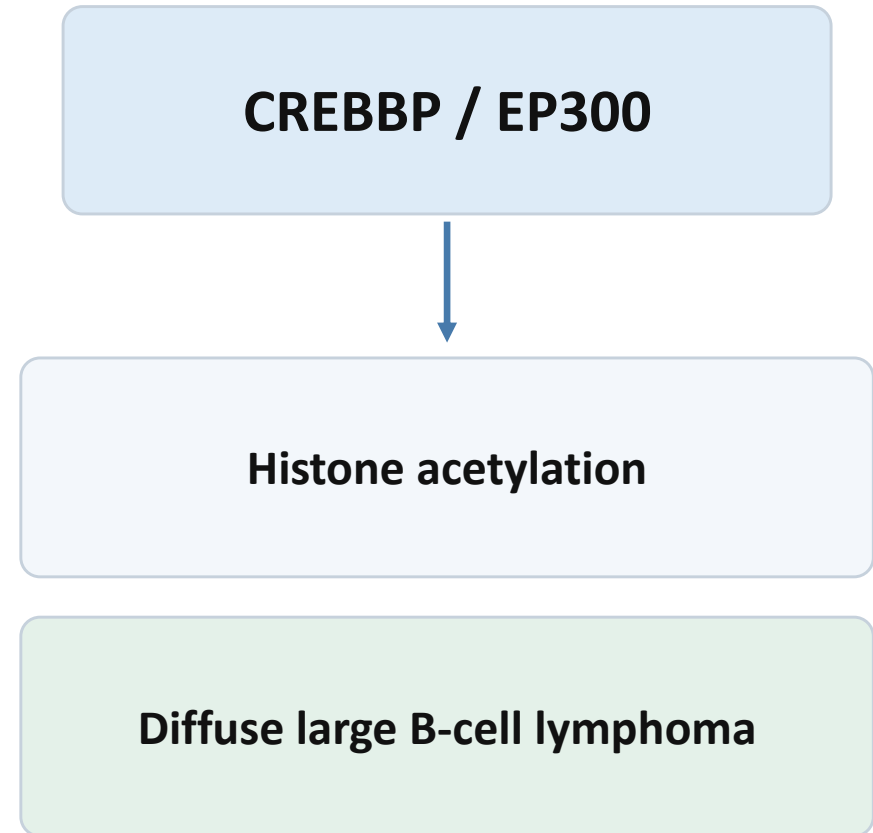
KMT2D links histone methylation to follicular lymphoma

- KMT2D encodes a regulator of histone methylation.
- It is listed as mutated in follicular lymphoma.
- The disease association is an example rather than a universal marker of all lymphomas.



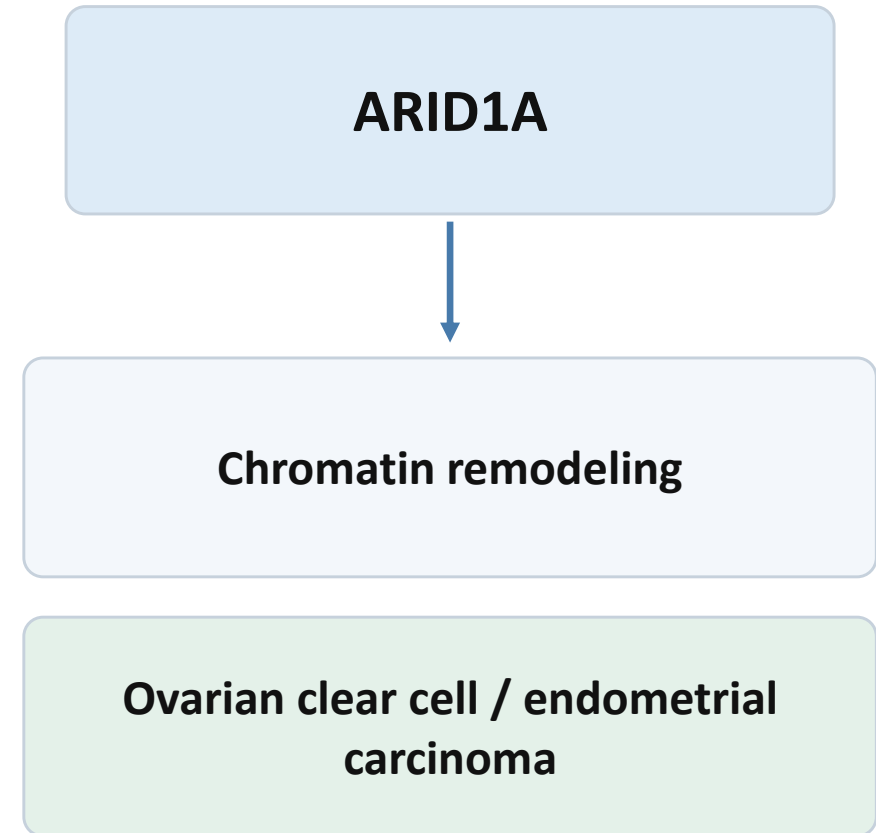
CREBBP and EP300 connect acetylation machinery to lymphoma

- CREBBP and EP300 appear among histone-acetylation regulators mutated in cancer.
- Diffuse large B-cell lymphoma as an associated tumor.
- Enzyme dysfunction may modify lineage-appropriate gene expression programs.



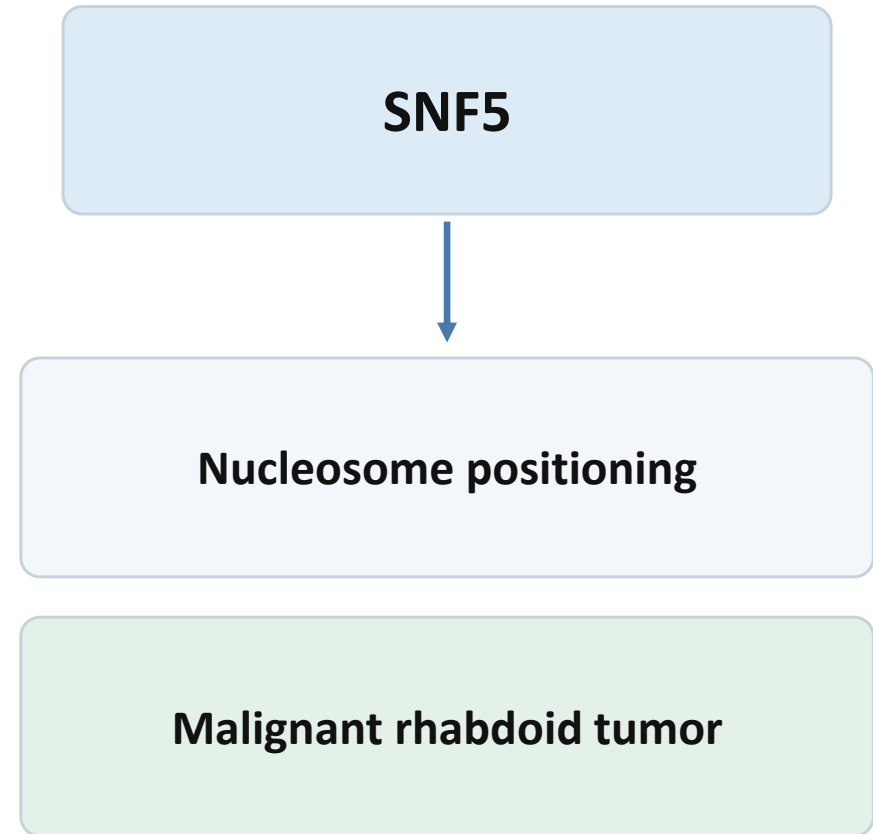
ARID1A illustrates disrupted nucleosome positioning

- ARID1A is involved in chromatin remodeling and nucleosome positioning.
- Its mutation with ovarian clear cell and endometrial carcinomas.
- The consequence involves chromatin accessibility rather than a direct DNA base change in target genes.



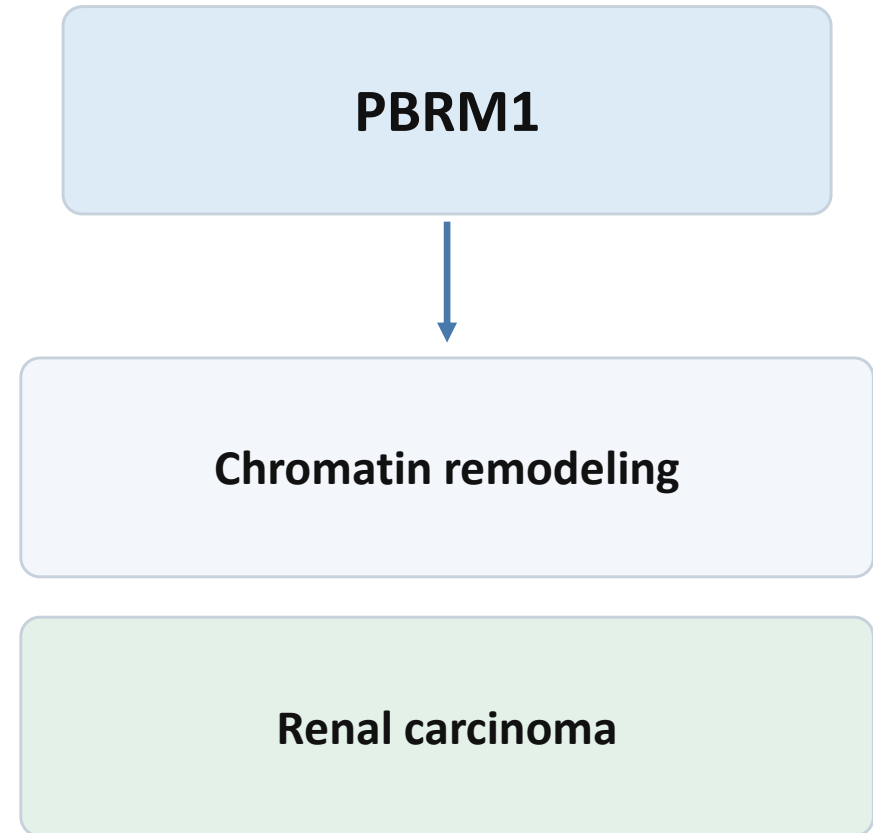
SNF5 links chromatin remodeling to malignant rhabdoid tumor

- SNF5 is included among nucleosome-positioning and remodeling regulators.
- Robbins Table 7.9 identifies malignant rhabdoid tumor as a characteristic association.
- This illustrates how a chromatin-regulator lesion may be central to a specific cancer.



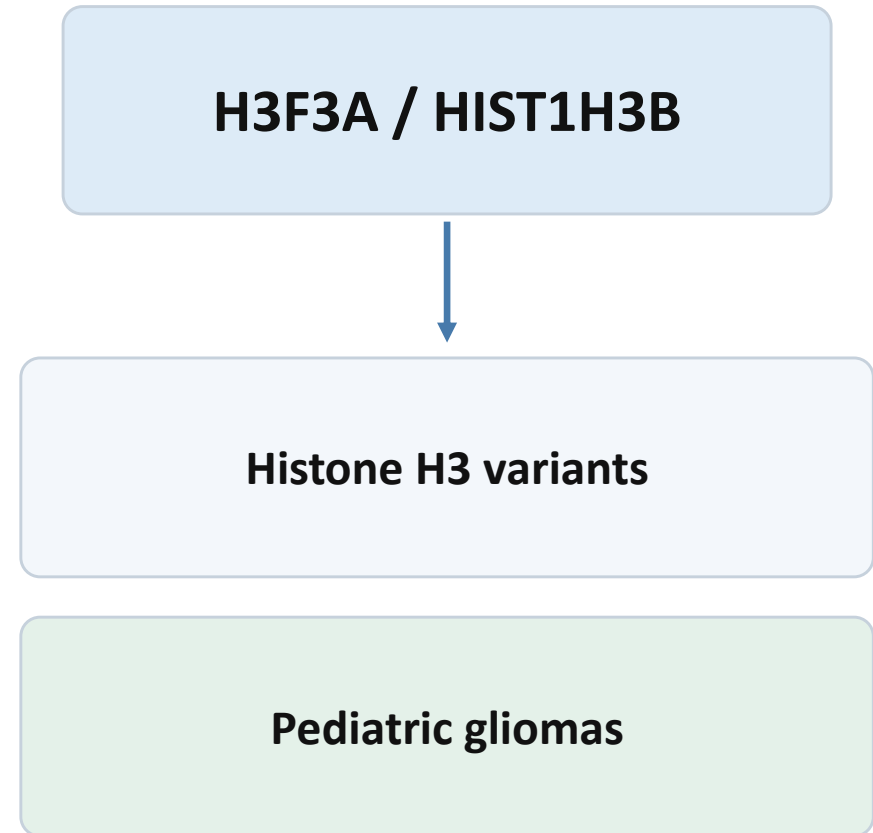
PBRM1 is a remodeling gene associated with renal cancer

- PBRM1 is included in the nucleosome positioning/chromatin remodeling group.
- Renal carcinoma is its example association in the Robbins table.
- This adds a second cancer lineage in which remodeling machinery is genetically altered.



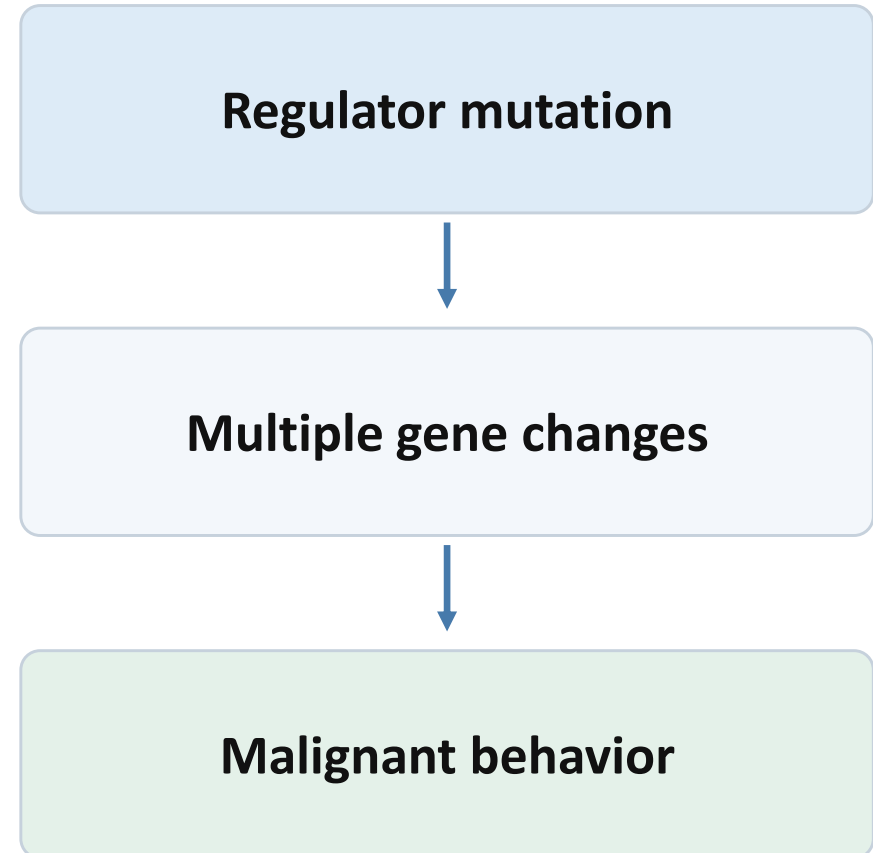
Cancer driver lesions can involve histones themselves

- The histone H3 variant genes H3F3A and HIST1H3B.
- These genes are associated with pediatric gliomas in Table 7.9.
- The affected component is a nucleosomal histone, not just a modifying enzyme.



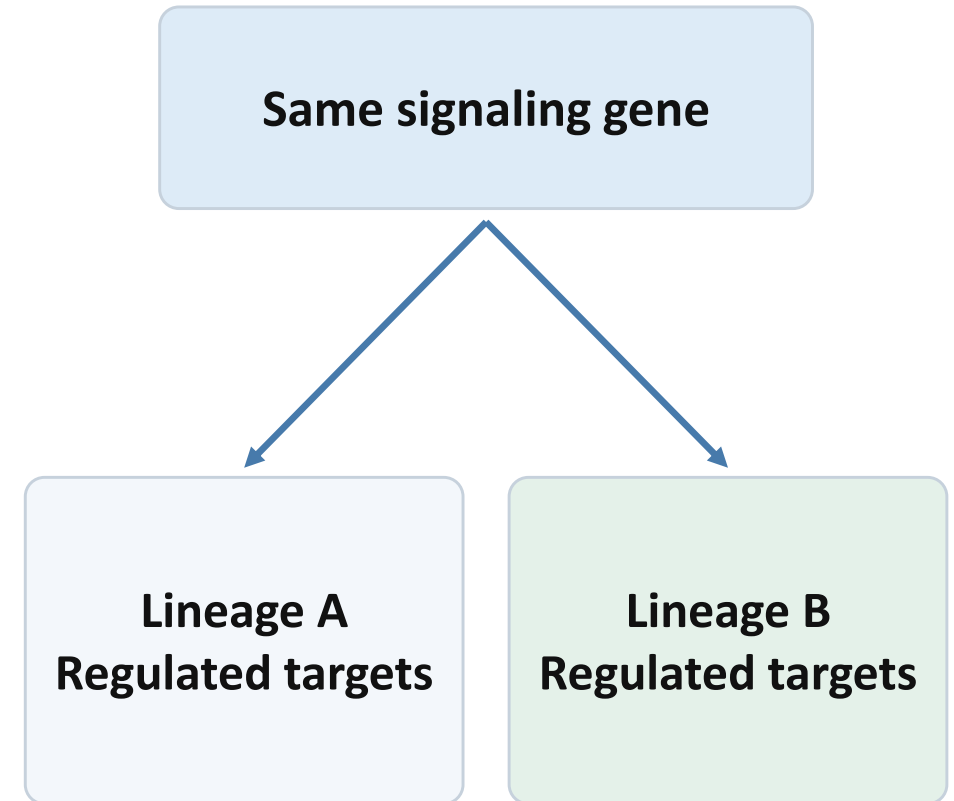
An epigenetic lesion may drive a network-wide phenotype

- A mutated chromatin regulator can alter the expression of many targets.
- Consequences may contribute to growth, differentiation and self-renewal abnormalities.
- A regulatory mutation should not be equated with a single, invariant downstream effect.



Cell lineage restricts the oncogenic effect of some genes

- Normal lineage identity depends on a cell-specific epigenetic expression program.
- An oncogenic lesion acts within that existing regulatory context.
- The same gene can therefore have distinct cancer effects in different tissues.



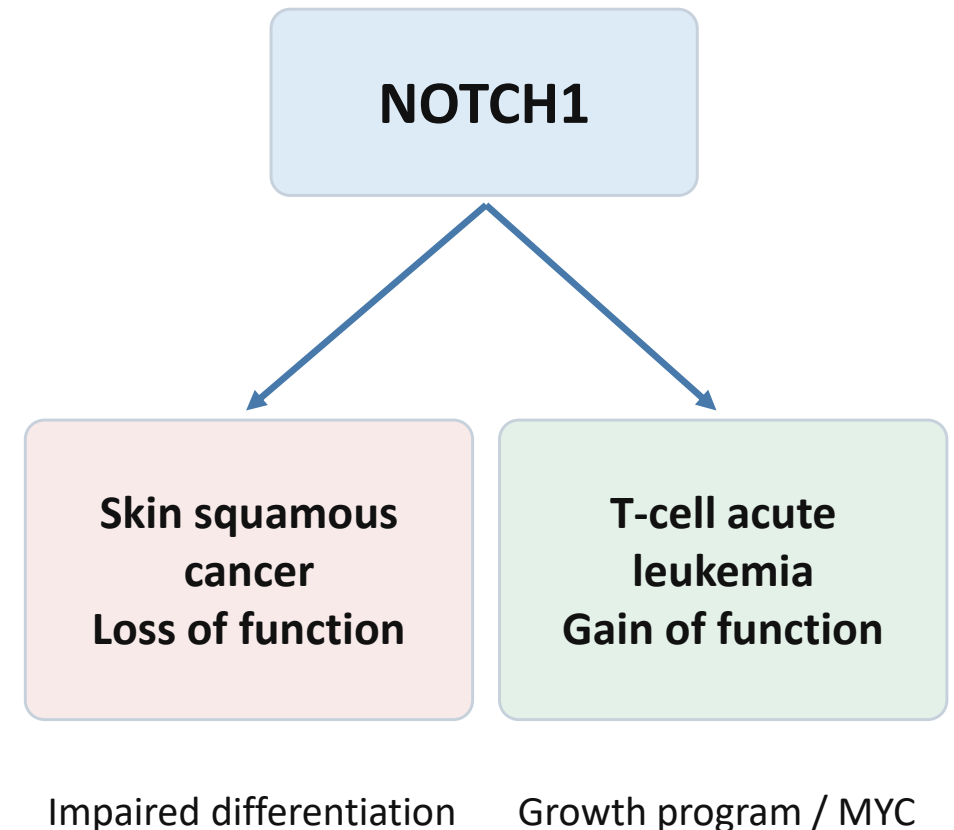
Some cancer genes are broad; Others are lineage-restricted

- RAS, MYC and TP53 are altered or dysregulated across many cancer types.
- These with lineage-restricted associations such as RB, VHL and APC.
- Epigenetic context helps explain why particular genes matter in particular cell identities.

Pattern	Examples	Interpretation
Broadly involved	RAS, MYC, TP53	Many tumor lineages
Lineage-restricted	RB, VHL, APC	Specific cellular context
Mechanistic principle	Epigenetic state	Controls target availability

NOTCH1 demonstrates context-dependent cancer behavior

- NOTCH1 to show that the same signaling family has divergent oncogenic roles.
- Its tumor-suppressive or oncogenic effect depends on tumor lineage and mutation type.
- The principle follows from the different target programs available in different cells.



NOTCH1 loss promotes cutaneous squamous carcinogenesis

- In cutaneous squamous cell carcinoma, NOTCH1 can be a tumor suppressor.
- Loss-of-function mutations impair differentiation.
- This does not imply that all NOTCH signaling is growth-suppressive in every tissue.

NOTCH1 loss of function



Impaired differentiation



Squamous carcinogenesis

NOTCH1 activation promotes T-cell acute lymphoblastic leukemia

- In T-cell acute lymphoblastic leukemia, NOTCH1 is a recurrent oncogene.
- Gain-of-function mutations drive pro-growth transcription, including MYC.
- This effect contrasts sharply with NOTCH1 loss in skin squamous carcinoma.

NOTCH1 gain of function



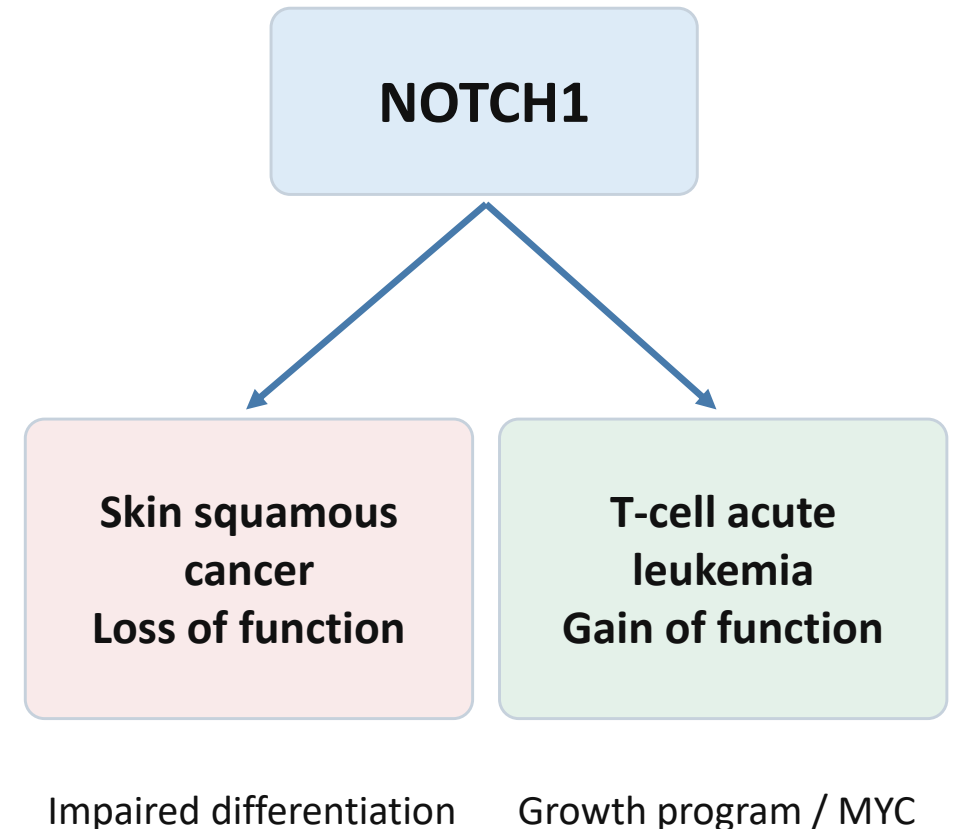
Pro-growth program / MYC



T-cell acute leukemia

The NOTCH1 example separates gene identity from gene effect

- The gene name alone does not determine whether a lesion is oncogenic or suppressive.
- Cell lineage and the type of mutation determine biological consequences.
- The underlying epigenomic environment helps define responsive transcriptional targets.



Cancer epigenetics also influences differentiation and self-renewal

- Epigenetic programs help maintain normal lineage identity and maturation.
- Cancer-associated changes may disturb differentiation and patterns of self-renewal.
- These properties contribute to the malignant phenotype beyond proliferation alone.

Altered epigenetic program



Changed cell identity



Malignant phenotype

Reversible enzymatic marks create therapeutic opportunities

- Many epigenetic states are maintained by enzymes that can be inhibited.
- Unlike fixed base-sequence mutations, some epigenetic marks are reversible.
- Drug development targets modifiers of histones, DNA methylation and other chromatin processes.

Reversible epigenetic modification



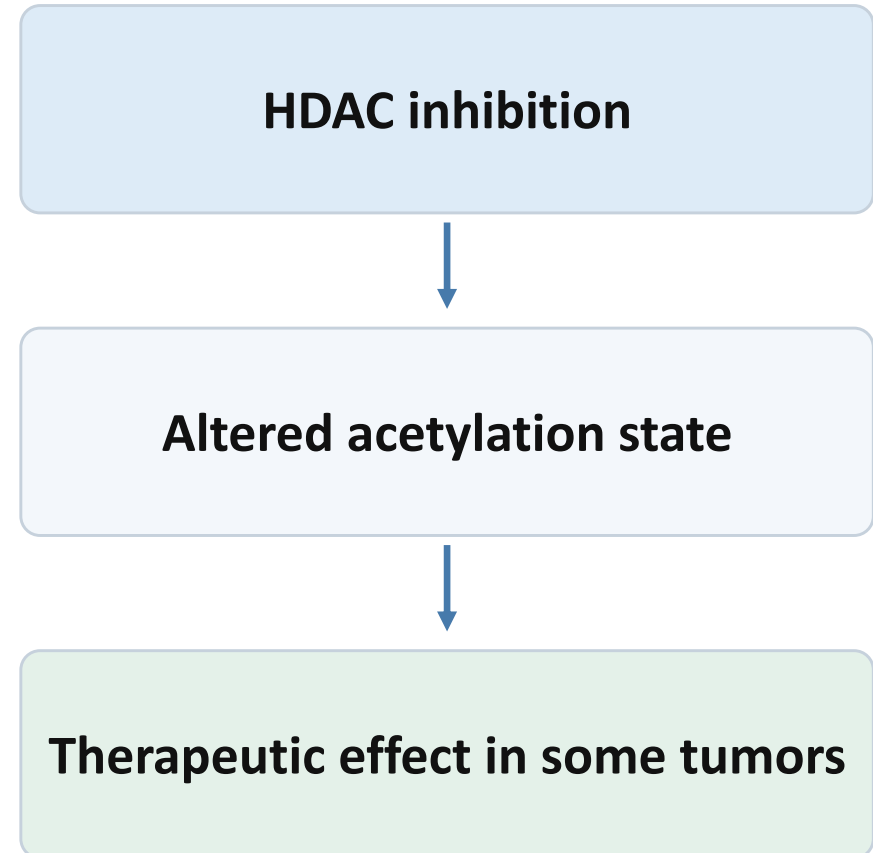
Enzyme can be pharmacologically targeted



Potential restoration of gene regulation

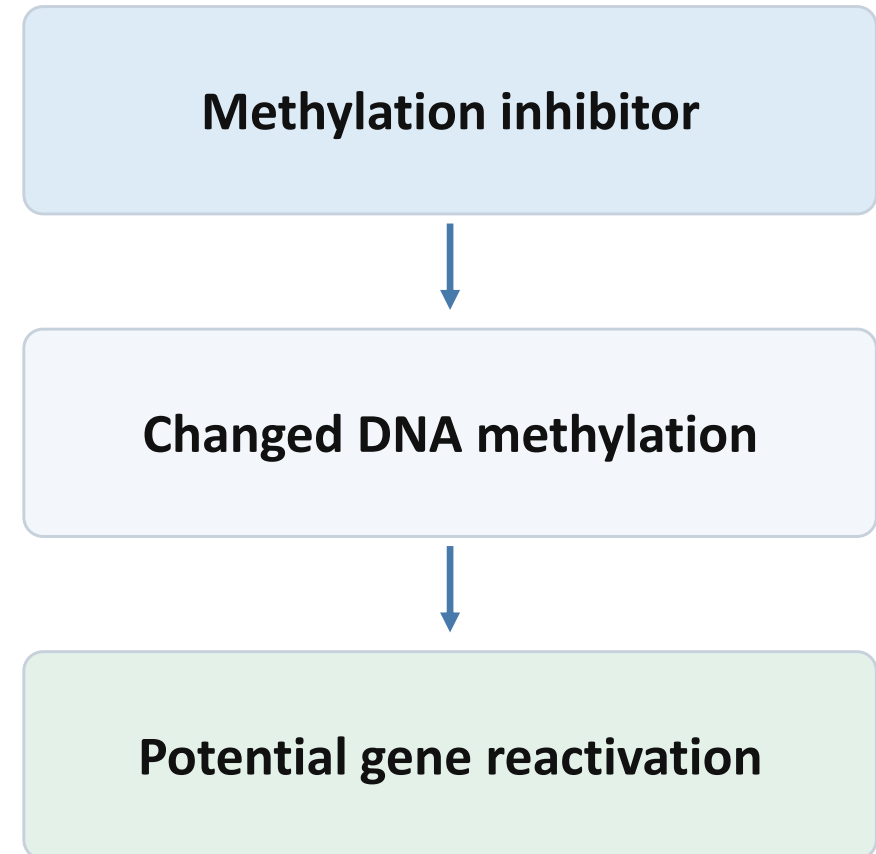
HDAC inhibitors exploit reversible histone acetylation

- HDACs remove acetyl groups from histones.
- Inhibitors of HDACs are approved for certain lymphoid tumors,.
- The rationale is interference with an enzyme that helps sustain aberrant chromatin states.



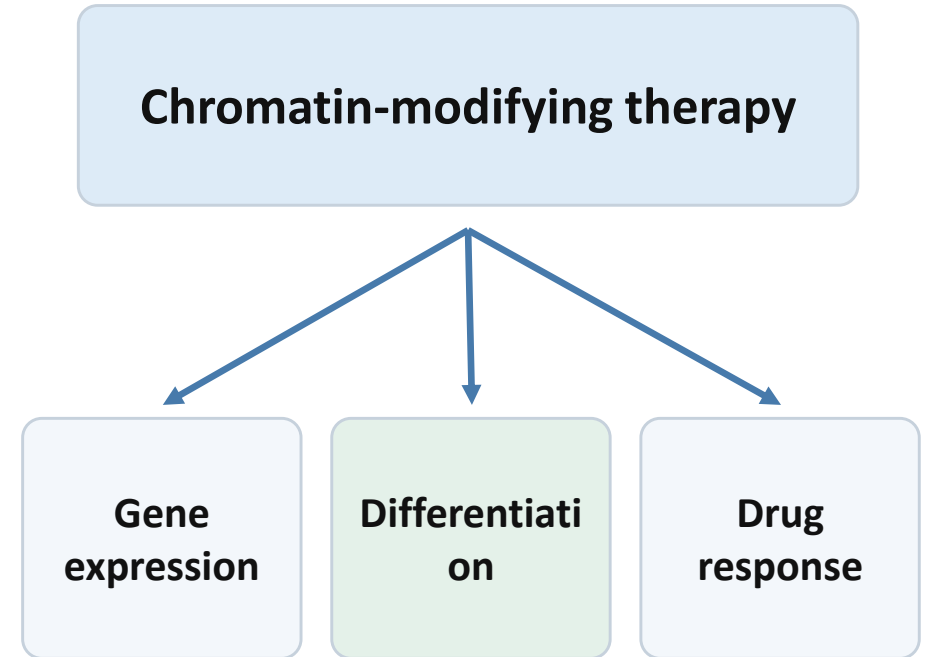
DNA methylation inhibitors are used in myeloid tumors

- DNA methylation inhibitors as therapies for myeloid tumors.
- Part of the rationale is possible reactivation of epigenetically silenced tumor suppressors.
- The proposed molecular rationale should not be equated with a proven mechanism in every patient.



Epigenetic drug action is not always gene-specific

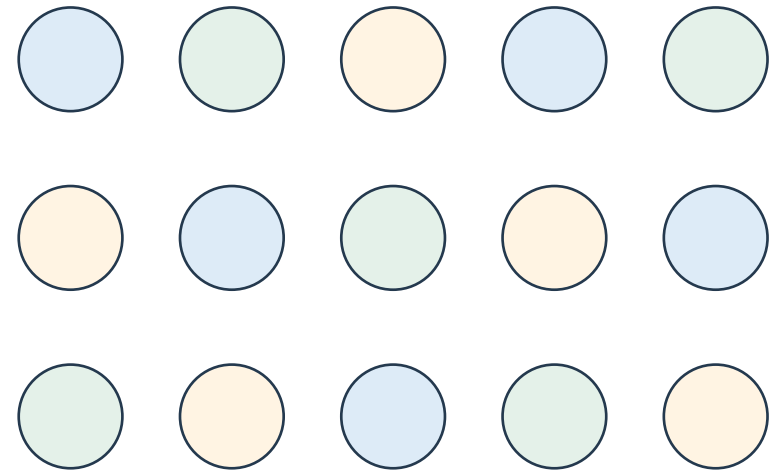
- Methylation and histone regulators may influence many genomic loci.
- Effects can therefore extend beyond any one silenced tumor-suppressor gene.
- This complexity limits simplistic predictions based on a single epigenetic mark.



Schematic: Interacting downstream outcomes

Intratumoral epigenetic heterogeneity adds another variable

- Cancer cells in a single tumor can differ in their epigenetic states.
- This diversity may arise alongside underlying genetic heterogeneity.
- Distinct regulatory programs can produce different responses to a common drug exposure.



Different regulatory states within one tumor

Epigenetic heterogeneity can produce drug tolerance

- Not all treatment-resistant cells necessarily carry a new fixed DNA mutation.
- An altered, potentially reversible state may reduce drug sensitivity.
- This state-based route to resistance complicates treatment strategies.

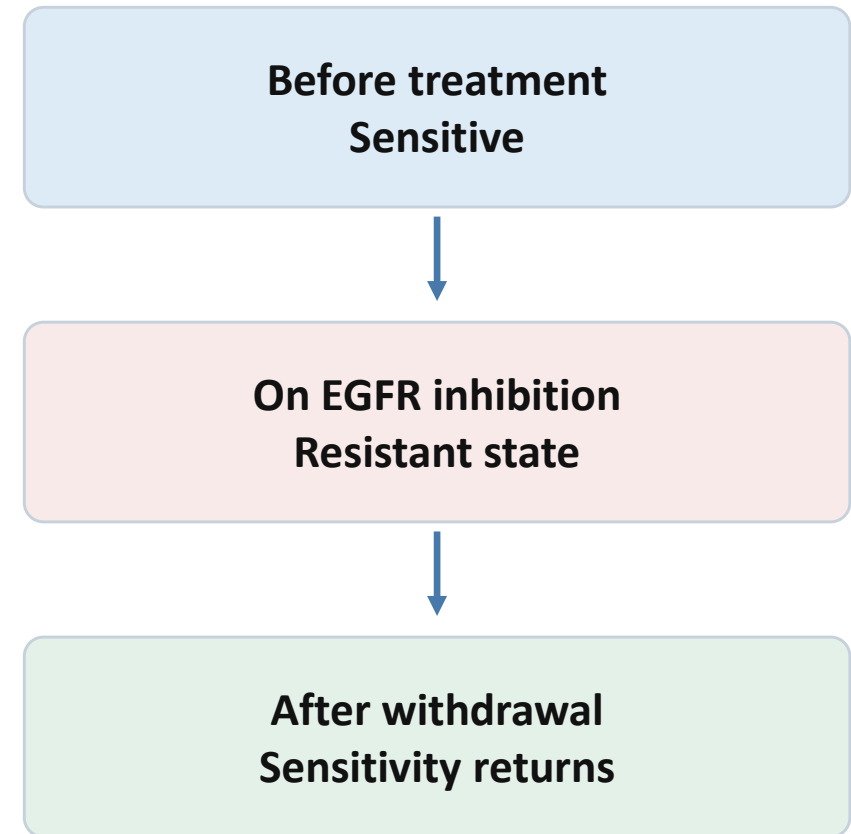
**Genetic
resistance
Stable sequence
lesion**

**Epigenetic
tolerance
Potentially
reversible state**

Distinct mechanisms • possible convergence

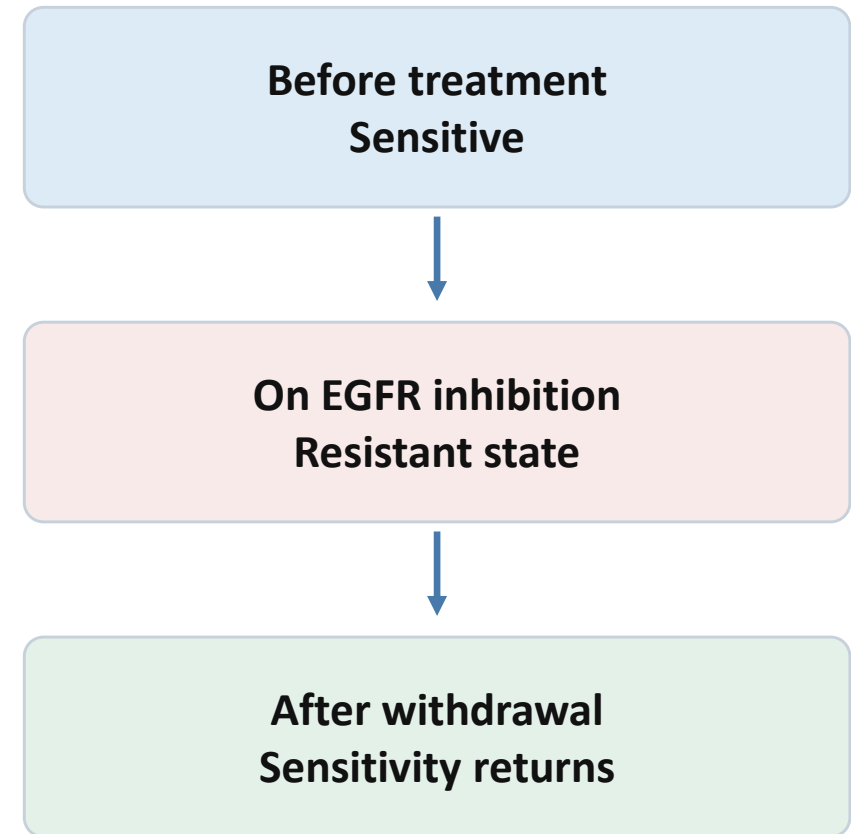
EGFR-inhibited lung cancer provides a reversible example

- Lung cancer cells that become resistant to EGF receptor inhibition.
- Epigenetic changes can contribute to the resistant phenotype.
- When inhibitor exposure stops, the cells may recover their previous inhibitor-sensitive state.



Drug withdrawal can reveal phenotypic reversibility

- A resistant state present during treatment does not always persist after drug removal.
- Reappearance of drug sensitivity supports a reversible component in the response.
- It is not evidence that all resistance mechanisms in lung cancer are epigenetic.



Resistance mechanisms must be interpreted mechanistically

- Genetic selection and epigenetic state change can both influence tumor evolution.
- Only some drug-resistant phenotypes are reversible.
- Phenotypic reversal is useful mechanistic evidence but does not identify a unique histone or DNA mark.

Question	Genetic route	Epigenetic route
Primary alteration	Sequence change	Regulatory state change
Potential reversal	Often fixed in clone	May reverse when context changes
Evidence needed	Genomic studies	State and functional studies

Clinicopathologic interpretation uses multiple evidence levels

- Morphology can reveal chromatin disturbance but not its specific molecular cause.
- Molecular data identify lesions in chromatin-regulatory genes or histone variants.
- Functional findings establish whether a regulatory state changes phenotype or drug response.

Morphologic observation



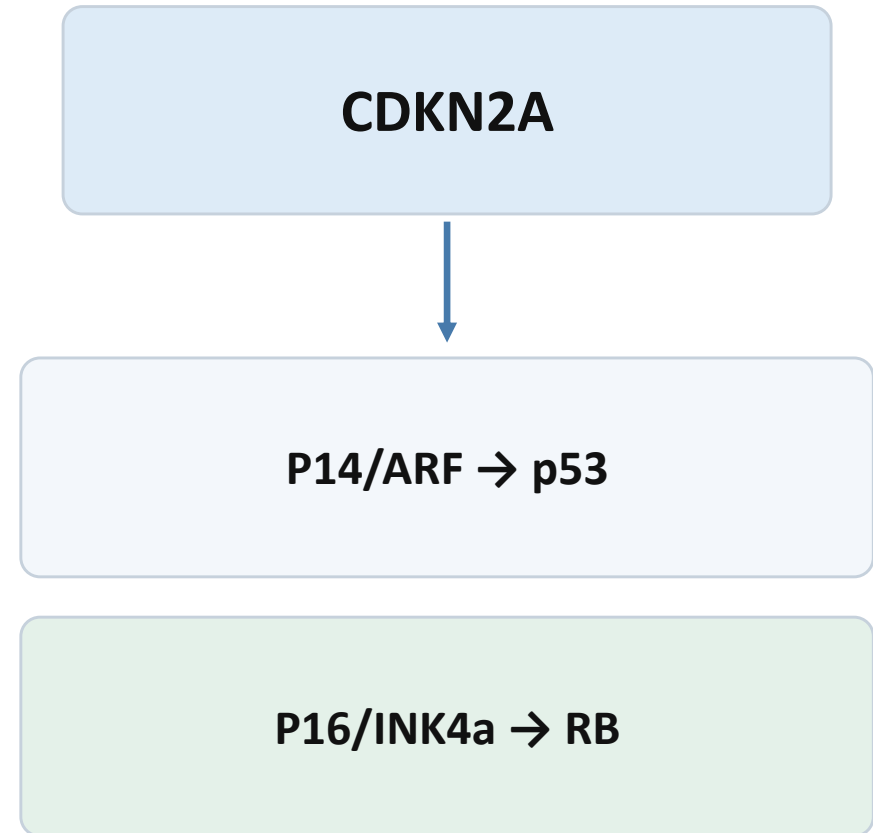
Molecular characterization



Functional interpretation

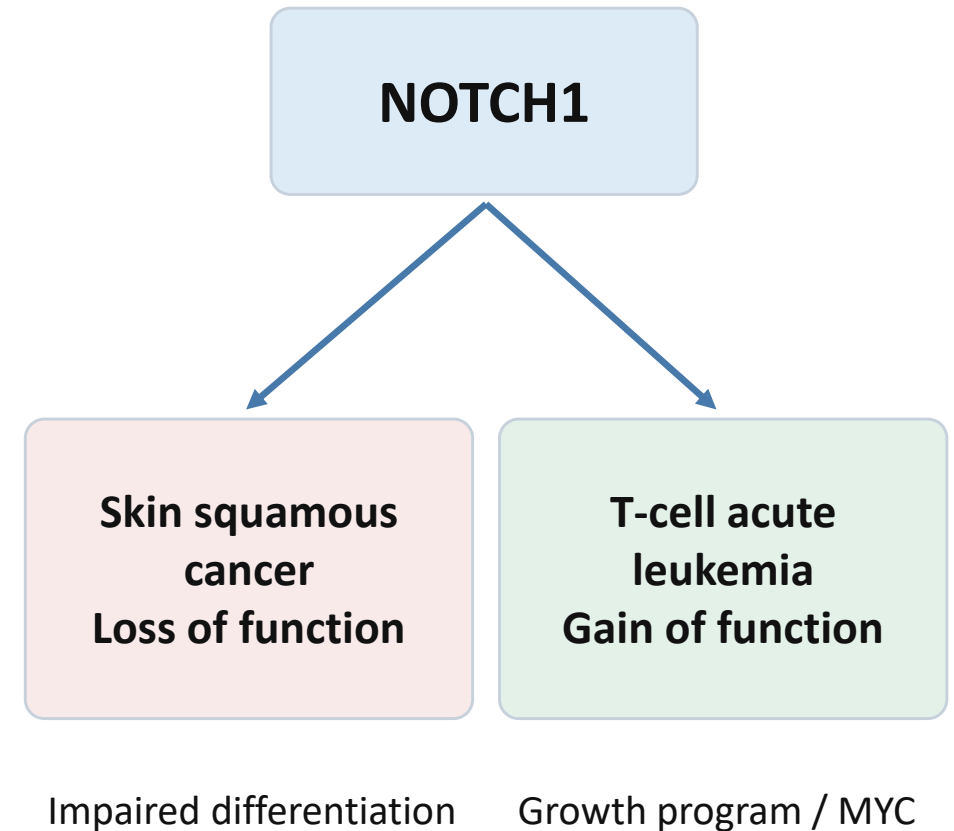
Case application: Loss of CDKN2A expression

- A tumor has reduced CDKN2A transcription with promoter hypermethylation.
- Question: Which two downstream tumor-suppressive signaling axes may be affected?
- Answer: P14/ARF–p53 and p16/INK4a–RB; Verify other-allele status separately.



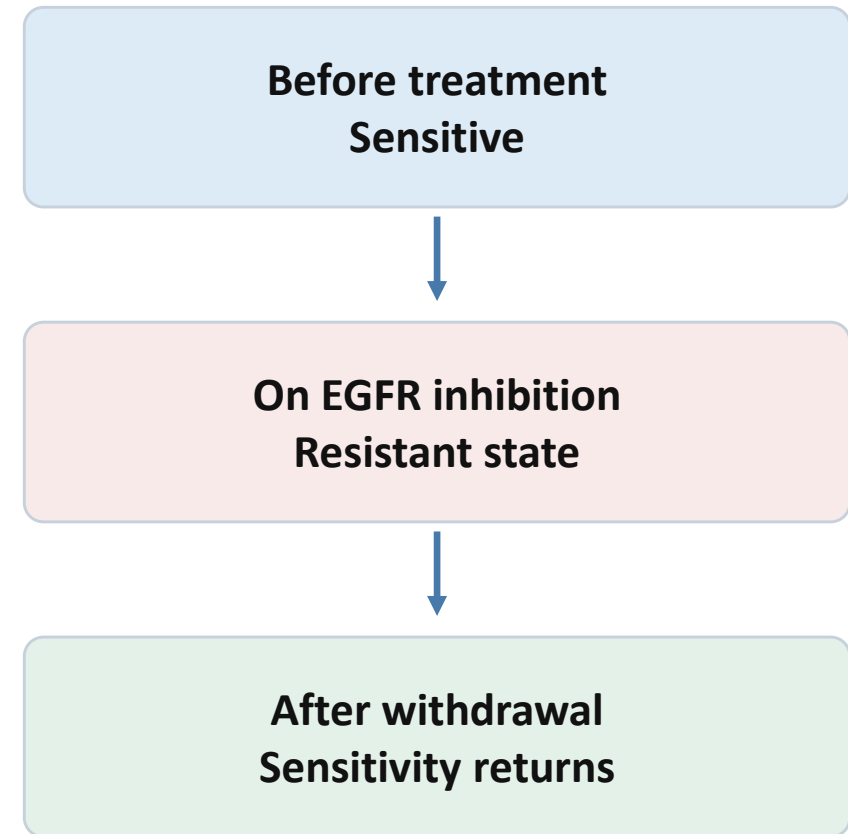
Case application: Opposite NOTCH1 roles

- One lesion shows NOTCH1 loss in cutaneous squamous carcinoma.
- Another shows NOTCH1 activation in T-cell acute lymphoblastic leukemia.
- Interpretation: Lineage-specific target programs allow suppressive versus oncogenic effects.



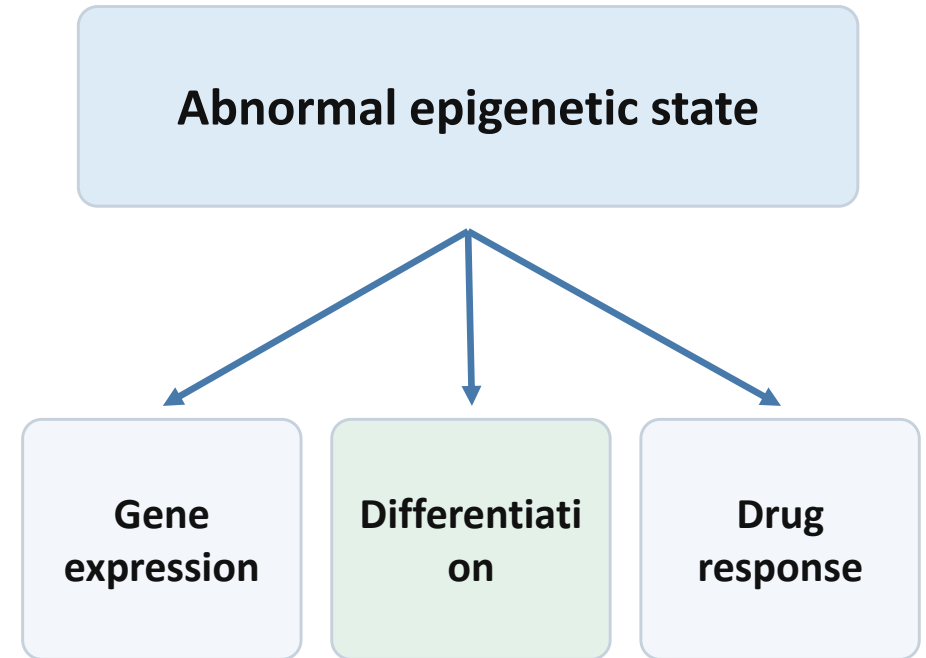
Case application: Transient drug resistance

- An EGFR-inhibited lung cancer population becomes drug-tolerant.
- Sensitivity returns after the inhibitor is withdrawn.
- This behavior is consistent with a reversible epigenetic contribution; It does not exclude genetic heterogeneity.



Integrated model: Genotype, epigenome and tumor behavior

- Mutations in epigenetic regulators can modify chromatin state and transcription.
- Cell lineage determines which target programs are biologically accessible.
- Epigenetic heterogeneity may modify drug sensitivity within a tumor.



Schematic: Interacting downstream outcomes

Core distinctions to retain

- Promoter hypermethylation can inactivate a tumor suppressor; Genome-wide methylation changes are more heterogeneous.
- Histone marks, nucleosome movement and enhancer loops regulate gene access.
- Cancer-associated mutations target chromatin regulators and sometimes histone proteins themselves.

Mechanism

Cancer phenotype

Clinical interpretation

Clinical implications of the epigenetic model

- Chromatin abnormalities have morphologic, transcriptional and therapeutic relevance.
- Epigenetic reversibility provides drug targets yet may enable transient resistance.
- Interpret each gene lesion in the lineage and molecular context in which it occurs.

Mechanism

Cancer phenotype

Clinical interpretation