Module-9
Lecture-6

Epigenetics and cancer

Introduction

In the human genome we have about 20-30,000 genes

Tissue specific gene expression is known to contribute for the identity of that tissue

Earlier we learnt that Mutations, deletions, etc lead to loss of expression of tumor suppressor genes in a tumor

Epigenetic silencing of tumor suppressor genes is another way, perhaps a more prevalent one, that accounts for the loss of expression of tumor suppressor genes in cancer

What is epigenetics?

Genetics could not explain all the differences between phenotype and genotype in some of the processes such as differentiation

For instance genetics alone cannot account for drastic changes observed between undifferentiated and differentiated cells of the same tissue given the idea that these cells have the same set of genes or genetic information.

Hence it was thought that the undifferentiated cells undergo a crisis first that determines their fate

This crisis was not dependent on their genes and so it was thought that this happens in addition to genes (in Greek it is epigenetic)

What is epigenetics?

Conrad Waddington in 1939 first coined the term “epigenetics” (literally meaning “above genetics”)

Epigenetics means the study of mitotically and/or meiotically heritable changes in gene expression that occur without a change in DNA sequence mediated mainly through changes in DNA methylation and chromatin structure.

Human tumors exhibit an overall loss of DNA methylation, but also show specific patterns of hyper methylation at certain promoters

DNA packaging

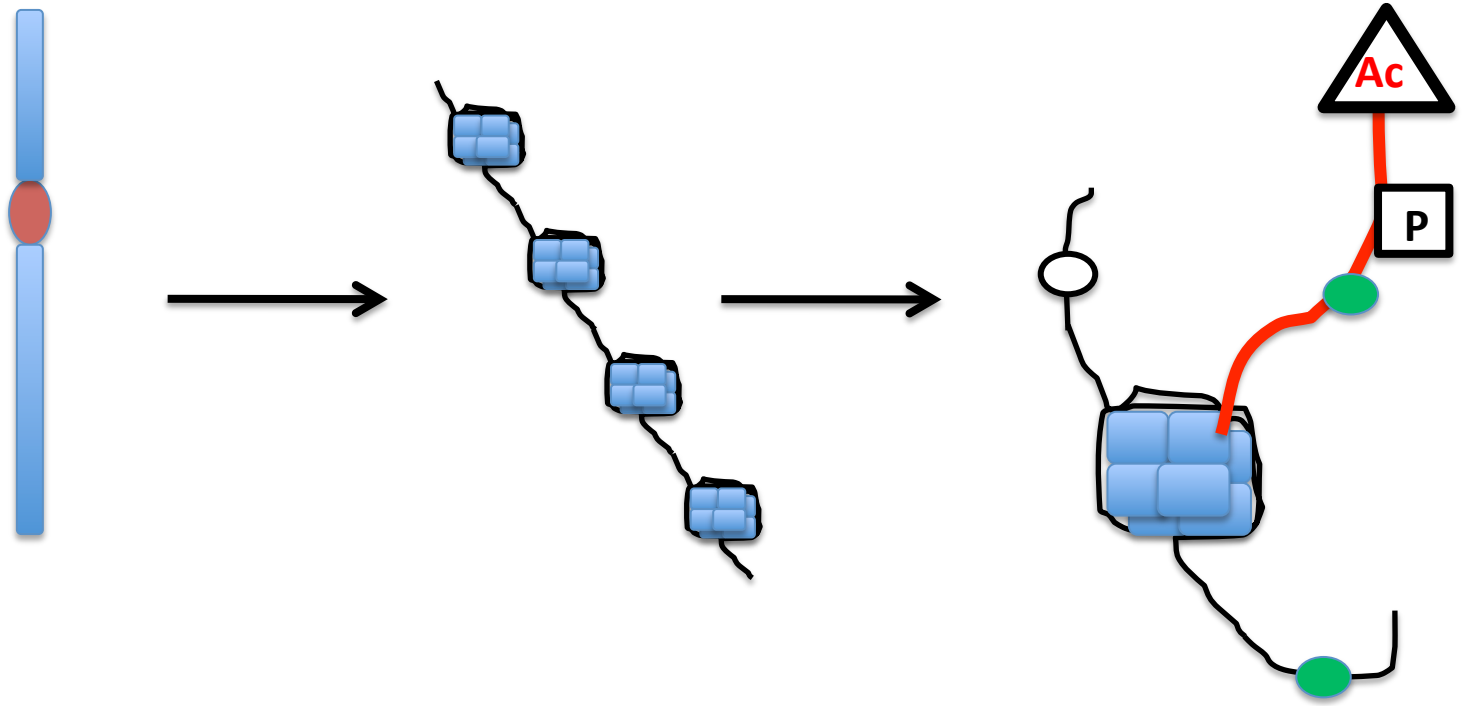
We have learnt about chromosomes, chromatin and nucleosomes

DNA packaging involves nucleosomes characterized by DNA chains (~146bp) wrapping around histone octamers

Histones are small (11–21 kDa) basic proteins that bind non-covalently with acidic DNA to form nucleosomes

Octamers are formed by H2A, H2B, H3 and H4 dimers

DNA methylation and Histone modifications



Chromosome Chromatin Nucleosome

Methylated residue

Unmethylated residue

P Phosphorylated residue

Ac Acetylated residue

Open and closed chromatin

Portions of DNA bound with histones and other proteins should be exposed for facilitating transcription factor binding

This influences the gene expression profile of any given cell type

Nucleosomes can be packaged into compact aggregates silencing transcription and thus constituting a closed chromatin

In contrast nucleosomes can also be arranged leaving some gaps for transcription factors to bind constituting an open chromatin

Thus the chromatin is said to be bivalent

Post-translational modification of key amino acid residues of the histones is important in this connection

These modifications constitute the so called “histone code” for the regulation of gene expression

Histone modifications

Histones are not just only involved in ‘DNA-packaging’

They undergo several modifications important for gene regulation

Covalent modification of histones includes acetylation of lysines,
methylation of lysines and arginines,
Phosphorylations of serines and threonines,
ADP-ribosylation of glutamic acids, and
ubiquitination and sumolyation of lysine residues

The status of acetylation and methylation of specific lysine residues contained within the tails of nucleosomal core histones is known to have a crucial role in regulating chromatin structure and gene expression

Histone modifications

Acetylation of some residues, such as lysine 9 of histone H3 (H3K9acetyl), by histone acetylases (HATs) usually signifies transcriptionally active regions

Deacetylation at these sites, mediated by histone deactylases (HDACs) usually signifies transcriptional repression.

Histone tail methylation can be activating or inactivating transcription depending on the sites.

H3K4 methylation denotes transcriptionally active areas while H3K9 methylation or H3K27 methylation suggests transcriptionally repressed regions

Histone methyltransferases place these methylation marks (Writers of the code)

Histone demethylases remove the marks (Erasers of the code)

Methylation

Well known epigenetic modification in humans is the cytosine methylation of DNA within the dinucleotide CpG

"CpG" is used to refer to both methylated and unmethylated dinucleotides

"p" refers to the phosphate moiety that connects deoxycytidine and deoxyguanosine

CpGs occur in the genome at a lower frequency because methylated cytosines can spontaneously deaminate to form thymine

DNA repair machinery does not recognize this leading to the accumulation of C-T mutations during evolution

Thus "99%" of the genome is CpG depleted and 1% is composed of discrete regions that have a high (G+C) and CpG content

Methylation

DNA methylation occurs along with other epigenetic modifications.
It is connected closely with the formation of nuclease-resistant chromatin

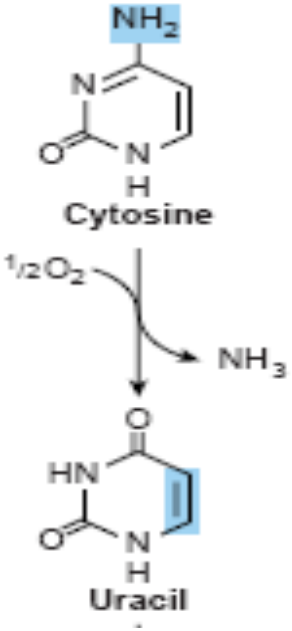
Methyl-CpG-binding proteins and DNA methyltransferases (DNMTs) are associated with histone deacetylases and histone methyl transferases

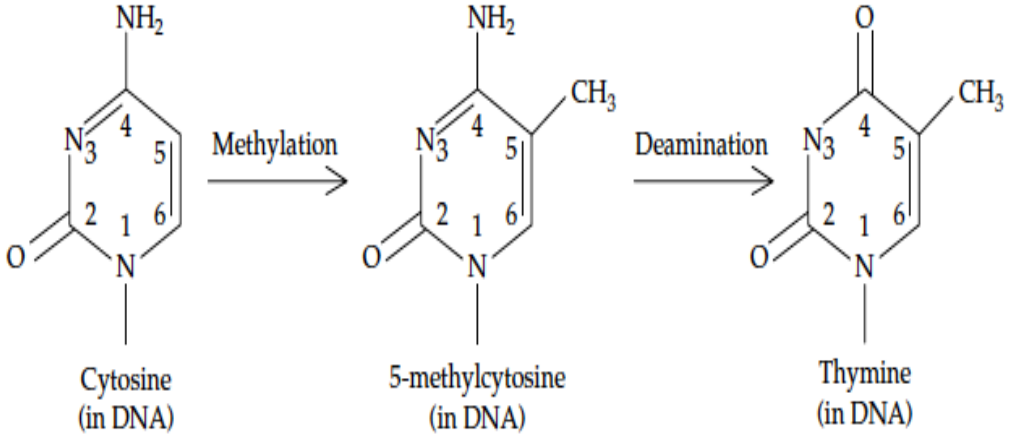
CpG-rich regions are known as **CpG islands**

Regions of comparatively low CpG density close to CpG islands are called CpG island '**shores**'

In **cancer cells**, the transcriptional silencing of tumour-suppressor genes occurs by CpG-island-promoter hypermethylation

Deamination and Methylation

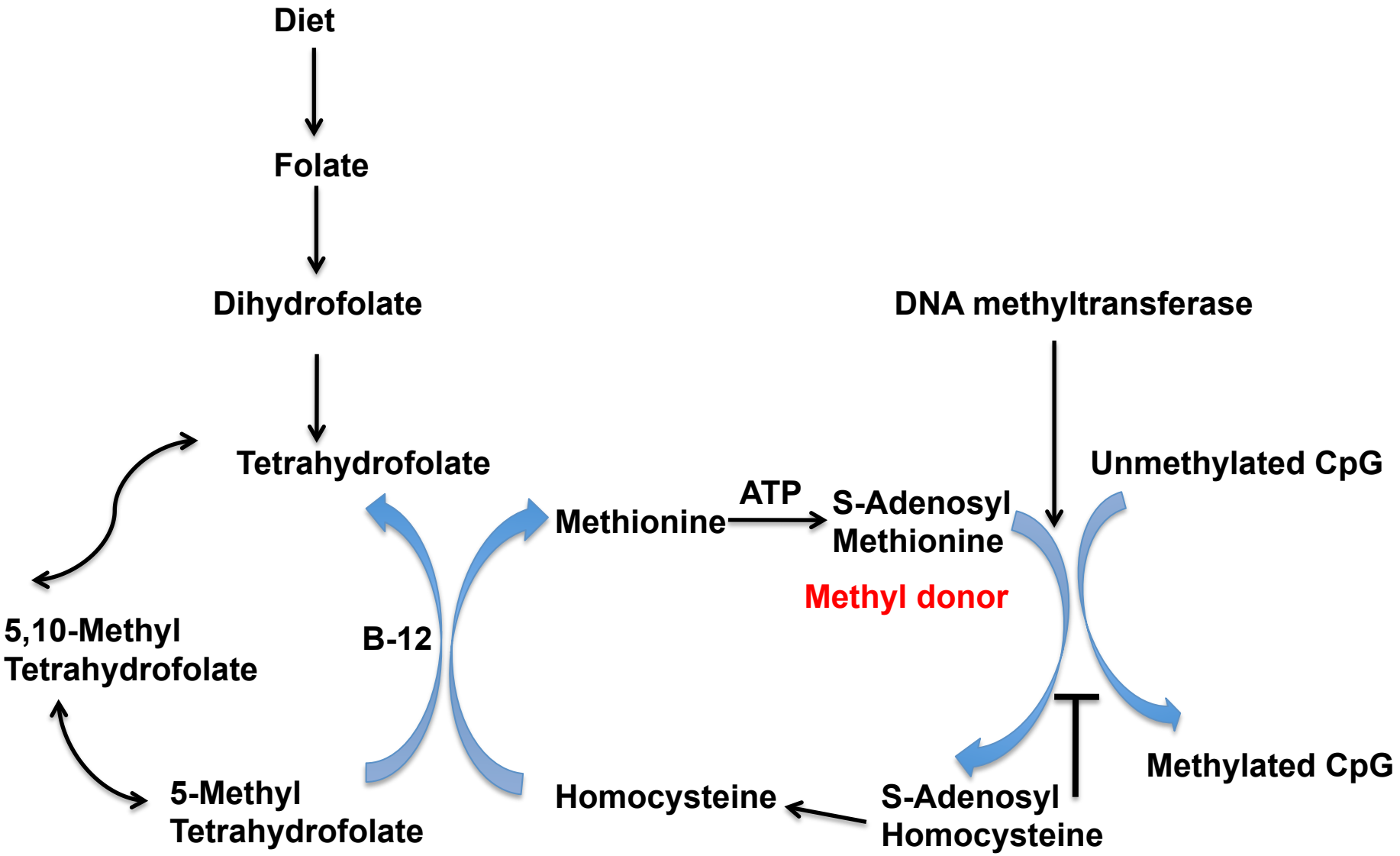




Cytosine upon oxidative deamination changes into uracil. This will be considered a mutation and hence will be removed by the DNA repair system and that is why we have Thymine but not uracil in DNA

Methylated cytosine upon oxidative deamination changes into thymine

Source of methylation



Methylation

CpG sites are concentrated either in CpG islands, located in approximately 60% of human gene promoters, or in regions of large repetitive sequences (for example, centromeres and retrotransposon elements)

Unmethylated clusters of CpG pairs are located in tissue specific genes and in essential “housekeeping” genes (involved in routine maintenance roles and are expressed in most tissues)

- There are specific proteins that bind to unmethylated CpGs and initiate gene transcription

Methyl groups are acquired through the diet and are donated to DNA through the folate and methionine pathways with S-adenosyl methionine being the donor of methyl groups that is covalently attached to the cytosine

- Low dietary levels of folate, methionine or selenium can affect methylation
- Such defects are associated with neural tube defects, cancer and atherosclerosis

Effects of Methylation

Thus imbalances in dietary nutrients can cause hypomethylation

This may lead to improper gene expression and genetic instability (chromosome rearrangements)

A common polymorphism in the methylene tetrahydrofolate reductase (MTHFR) gene (MTHFR 677CT) increases the risk of breast cancer up to 3-fold in premenopausal women

Effects of Methylation

Repetitive genomic sequences are heavily methylated.

DNA methylation protects chromosomal integrity, by preventing chromosomal instability, translocations and gene disruption

Genomic imprinting requires DNA hyper methylation at one of the two parental alleles of a gene in order to establish mono allelic expression
Loss of imprinting is a mechanism of gene activation in some types of cancer

Gene-dosage reduction is observed in X-chromosome inactivation in females

DNA methylation is needed for the germ line-specific expression of genes

Methylation is a mechanism for silencing tissue-specific genes in cell types in which they should not be expressed

Changes in DNA methylation in cancer

There is an overall decrease in the levels of DNA methylation in cancers

The loss in methylation is evident within repeat sequences, and promoters of selected genes, and in the pericentromeric regions of chromosomes

These changes are linked with abnormal gene expression and chromosomal instability

Promoter DNA hypermethylation is observed more frequently in cancers than mutations

This is important since it is a known mechanism for loss of tumor suppressor gene function

The genes affected include those involved in
cell adhesion – cadherin
DNA repair – MLH1
Cell migration – TIMPs
Differentiation – TGF beta receptor

Hypermethylation in human cancers

Cancer	Hypermethylated gene
Breast, Ovary	BRCA1
Cervix	p16
Kidney	TIMP-3
Lung	P16, p73
Prostate	BRCA2
Pancreas	APC
Uterus	hMLH1

Interactions between DNA methylation and chromatin changes

H3K9 methylation, and the histone methyltransferases are required for DNA methylation in lower organisms such as *Arabidopsis and Neurospora*

Polycomb group of proteins which target another key gene repression mark to nucleosomes, H3K27me, have been implicated in the targeting and maintenance of DNA methylation

Methyl cytosine binding proteins (MBPs), and the protein complexes in which they reside, can bind to methylated CpG sites to help relay a silencing signal

These complexes contain histone deacetylases (HDACs), which catalyze the deacetylation of key amino acid residues, such as H3K9, that are highly characteristic of transcriptionally silent regions of DNA

Interactions between DNA methylation and chromatin changes

The DNMTs themselves also interact with HDACs

This helps to target these enzymes to sites of DNA methylation

Changes in cancer include
increases in the levels and activities of the DNA methylation catalyzing enzymes
or the proteins in complexes that modulate the enzymes that catalyze transcriptional repression histone modifications
or altered levels of the repressive histone marks

loss of acetylation at H4K16
and increased levels of H4K20 acetylation

Interactions between DNA methylation and chromatin changes

DNA methylation at gene promoters can result in gene silencing

Histone modifications associated with active gene transcription such as acetylation of H3K9 and H4K16 decrease

Histone modifications associated with transcriptionally repressive chromatin such as H3K9me2 and me3, and H3K27me3 increase

The enzymes involved in the repressive marks increase in their activities

H3K27me3 histone modification is an important indication in human embryonic stem cells for genes that would become hypermethylated in adult cancers

Additionally H3K9 me2 and the H3K27 me3 mark may be involved

Gene silencing and tumor progression

Although the loss and gain of methylation can occur at any of the multistages of cancer development, most often these happen at the initial stages

For instance loss of a tumor suppressor gene may give a survival advantage for a preneoplastic cell or progenitor or stem cells

Such a change may also confer the ability withstand adverse circumstances such as chronic inflammation, ROS, hypoxia etc and still make a progress towards carcinogenesis

Cells encountering DNA damage would normally undergo apoptosis but if these changes in methylation occur they may survive and expand and select for mutations or other changes that favor tumor progression

Gene silencing and tumor progression

p16 gene is a frequent target for early methylation in breast cancer and non-small cell lung cancer

Histologically normal mammary epithelium from some women without malignancy can harbor *p16* promoter hypermethylation

Experimentally, early loss of *p16* in mammary epithelial cells precedes genomic and epigenetic instability

In colon cancer aberrant crypt foci show DNA hypermethylation of a family of genes, the SFRPs, (secreted frizzled receptor proteins) that antagonize Wnt interaction with its receptors.

If this hypermethylation persists throughout colon tumor progression it may drive the Wnt pathway further in collusion with the downstream mutations

Translational aspects of epigenetic changes

Understanding on the epigenetic changes in cancer may provide potential biomarkers for use in cancer risk assessment, early diagnosis, and prognosis assessment

H3 and H4 modifications correlate with aggressiveness of prostate cancer

In several cancer aggressive behavior of cells correlate with increases in levels of DNA methyltransferases and histone methyltransferases such as EZH2 for H3K27 methylation, and other PcG (polycomb group) gene silencing constituents

Reversal of DNA hypermethylation and aberrant gene silencing are promising strategies to combat cancer

Development of molecular markers

One idea is to use promoter–hypermethylation sequences as a molecular signature (**as a biomarker**) for each type of cancer

Some studies with sputum DNA from patients at high risk for lung cancer have shown that invasive tumors may be predicted, more than a year before clinical detection of cancer

Abnormal methylation markers in sputum were useful for predicting recurrence of the disease in patients with surgically resected early stage lung cancers

p16 gene in DNA from lung cancers predicts poor outcome

In normal colon tissues gene promoter methylation increases with age and parallels the risk of cancer

Metastatic potential can be predicted on the basis of the E-cadherin promoter methylation in breast and oral cancers

Development of molecular markers

O^6-MGMT is a gene that encodes for a protein that mediates removal of bulky alkylation adducts from guanosines

Silencing of *O^6-MGMT* gene is observed in many cancers

Consequently these cancer cells cannot efficiently repair alkylation damage

Such cancer cells are sensitive to alkylating agents such as temozolomide

Thus brain tumors harboring *O6-MGMT* respond better to temozolomide

Strategies for cancer prevention and therapy

Gene silencing by epigenetic alterations is almost an alternate mechanism like mutations for the development of cancer

Hence we can exploit this mechanism for prevention and therapy

Of course mutation is not reversible but epigenetic changes are reversible

Since there are several changes we get the option of individual and combined targets for intervention

Since many changes occur during the early stages of cancer these are ideal for preventive measures

Reversal of gene silencing potentially can alter several signaling pathways making a significant contribution in managing the different stages of cancer

Strategies for cancer prevention and therapy

DNA methylation inhibitors

5-azacytidine
5-aza-2′-deoxycytidine (both are demethylating agents)

Efficacy of aza-cytidines correlates with the acute reversal of gene silencing

DNA methylation inhibitors (DNMTi) are usually the nucleoside analog family members

Inside the cells they are converted into deoxynucleotide triphosphates and are incorporated into replicating DNA in place of cytosine

I have mentioned about these drugs and HDAC inhibitors in another lecture on molecularly targeted drugs for cancer therapy

Strategies for cancer prevention and therapy

Similarly HDAC inhibitors are also already in the clinic

They generally have antitumor, growth inhibitory, proapoptotic, and prodifferentiation properties

They upregulate the expression of p21 (cell cycle inhibitor) due to hyper acetylation of its promoter by these drugs

Suberoylanilide hydroxamic acid (SAHA), also known as vorinostat, has been approved for the treatment of T cell cutaneous lymphoma

Other promising hydroxamic acid derivatives are Trichostatin A, Panobinostat and Belinostat

Apart from these some short chain fatty acids such as sodium butyrate, phenyl acetate, phenyl butyrate, valproate also act as HDAC inhibitors

Others include certain cyclic peptides and benzamide derivatives

Strategies for cancer prevention and therapy

Many other compounds are being developed as drugs either alone or in combination with other known drugs

Inhibitors of demethylases (HDMs)

Pargyline
Phenelzine
Tranylcypromine

Inhibitors of methyltransferases (HMTs)

Chaetocin
DZNep
Allantodapsone

Inhibitors of histone acetyltransferases (HATs)

Garcinol
Curcumin
Anacardic acid

Sirtuin (class III HDAC) inhibitors such as sirtinol are gaining importance as well since they induce p53 activity and thus induce apoptosis

Biogenesis of miRNAs

MicroRNAs (miRNAs) are noncoding RNAs (ncRNAs) of 18-25 nucleotides in length

They are fine regulators of gene expression and hence affect several biological processes such as development, differentiation, and cell cycle regulation to senescence and metabolism

miRNA genes are transcribed like other genes in the nucleus with RNA polymerase II

The primary transcript called pri-miRNA is very long with the cap and poly A tail etc similar to the protein-coding genes

They are further processed by a ribonuclease called Drosha and its partner DGCR8 to form pre-miRNAs

This is exported from the nucleus in the presence of exportin 5

Biogenesis of miRNAs

This is further cleaved by another ribonuclease called Dicer to form a duplex RNA that binds to a complex called RISC containing many proteins including the Argonaute family of proteins

One of the strands get separated and acts as miRNA while the other strand gets degraded. The mature miRNA binds with a complimentary sequence present on the 3'UTR of the target gene mRNAs

If there is a perfect complimentarity this results in the degradation of the target mRNA and if there is partial complimentarity it results in translational inhibition thus silencing the target gene expression in both cases

Interestingly some miRNAs are found to be oncogenic while many other miRNAs act like tumor suppressors

Epigenetic regulation of miRNAs in human cancer

Epigenetics plays an important role in the aberrant expression of miRNAs in many cancers

The expression levels of several miRNAs change upon treatment with HDAC inhibitors

miR-127 is epigenetically silenced by promoter hypermethylation and histone modifications in many cancer cells

Acute lymphoblastic leukemia patients have shown a signature expression of 13 miRNAs in the CpG islands and high levels of K9H3me2

5-aza-2'- deoxycytidine increased at least one miRNA in many ALL patients

miR-124a is methylated in many ALL patients, and its promoter hypermethylation is associated with a higher relapse rate and mortality rate

miRNAs as regulators of epigenetic effectors

miRNA 29 targets DNA methyltransferases in lung cancer cell lines

HDAC4 is a direct target of both miR-1 and miR-140

miR-449a binds to the 3'-UTR region of HDAC1

miR-101 directly targets EZH2 (enhancer of zeste homolog 2), a histone methyl transferase, in both prostate and bladder cancers

EZH2 is the catalytic subunit of the polycomb repressive complex 2 (PRC2) and is responsible for heterochromatin formation by trimethylating histone H3 lysine 27 (H3K27me3), leading to the silencing of several TSGs

We started this lecture with a definition for epigenetics

We discussed the open and closed chromatin structures and their effects on gene expression

We learnt about the histone modifications and the histone code hypothesis and how the code is written and erased by enzymes

We have learnt about DNA methylation, the source of methylation, the enzymes involved in methylation and demethylation and the effects of these changes on gene expression

Some examples were given about hypermethylation in cancers

Genesilencing and tumor progression, translational aspects of epigenetics, development of molecular markers, strategies for cancer prevention and therapy were also discussed

Finally some of the latest developments such as the epigenetic regulation of miRNAs in cancer and how miRNAs act as regulators of epigenetic effetors were also discussed

END