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Cross talk between Epigenetics and Human Disease-A multifaceted review

Debojyoti Dutta

*Assistant Professor, Department of Zoology, A B N Seal College, P O & Dt- Cooch Behar, PIN-736101, West Bengal Email-debojyotidutta2001@yahoo.com

ABSTRACT

This paper deals with few basic queries and their most possible explanation regarding epigenetic in human disease has been discussed. Gene mutation influences epigenetic diseases, but it has been noticed that certain environmental factors affect the epigenetic diseases. The food habits have direct influence on human epigenetic disease. After studying about the three common processes namely DNA methylation, his tone modification and micro RNA targeting influence gene expression. It is understood that turning gene on and off is easier than changing the DNA sequence. Many drugs have been approved are in use and also underdevelopment which can easily reverse the adverse epigenetic expressions in individuals even after being transmitted for one or several generations by certain procedures like DNMTs & HDACs inhibiting, immunotherapy, targeted therapy etc. Therefore, in near future large number of human diseases can be treated or rather cured by this innovative approach.

KEYWORDS :- Epigenetic, Cancer, Methylation, microRNA.

***Corresponding author**

Debojyoti Dutta

Assistant Professor,

Department of Zoology, A B N Seal College,

P O & Dt- Cooch Behar, PIN-736101, West Bengal

Email-debojyotidutta2001@yahoo.com

INTRODUCTION

The term 'Epigenetic' refers to influence on the heritable changes of gene expression without changing of DNA sequence**.** It shows involvement in the phenotypic changes only, not in the genotypic changes. There are three most common ways like DNA methylation (DNA hypermethylation & hypomethylation) , non coding RNA silencing and Histones modification can influence the heritable gene expression [2]. It is very necessary to understand how DNA methylation is implicated in the regulation of genomic imprinting. Now a day's attention is drawn to the scientific community on how altered DNA methylation can result in human diseases such as imprinting disorders and cancer. Research clearly gives the output that DNA methylation and resulted aberrations can contribute cancer either through hypomehylation or hypermethylation.

DNA hypomethylation influence the chromosomal instabilities and occur oncogene activation during oncogenesis. DNA hypermethylation leads to inactivation of tumor suppressor gene during tumerogenesis. Disruption of the balance of epigenetic networks can cause cancer, some neurological disease, autoimmune diseases, allergic disorders, human imprinting disorders. Histone acetylation or deacetylation are the most common histone modification, which involves a number of diseases such as neurological disorders and cancer [9] .

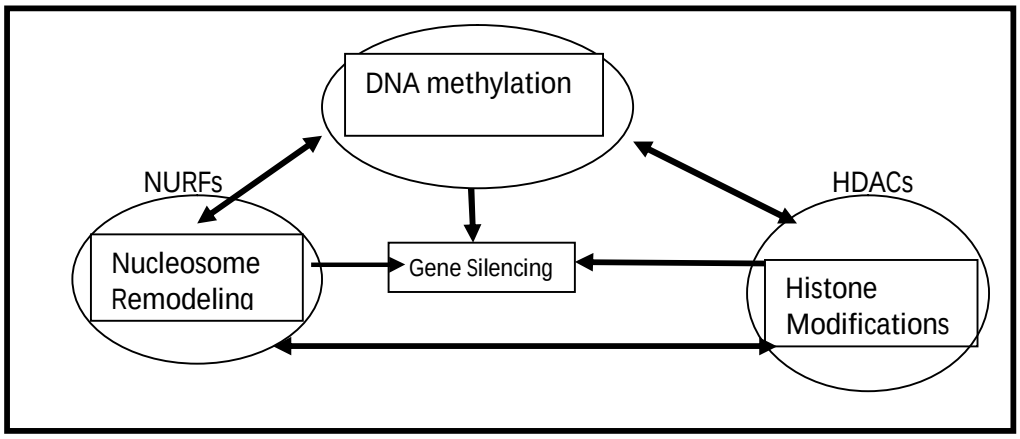

Fig.1. Simplified word diagram showing interrelationship of three basic epigenetics mechanism. (Abbreviation Used:-NURFs-Nucleosome Remodeling Factors**, HDACs-** Histone Deacetyleases **)**

In this review paper attempts are undertaken to address about following type of queries and their probable answers based on modern updated literature.

Technical Terminology Associated:-

- **RNA silencing** – RNA silencing or RNA interference (RNAi) is a biological process in which RNA molecules inhibit gene expression by neutralizing targeted mRNA molecules. RNA silencing also refers to a family of gene silencing effects by which gene expression is negatively regulated by non coding RNAs such as microRNAs.

- **Acetylation & Deacetylation -** Acetylation is the process which helps in unwrapping the DNA from histone with the aid of Histone Acetyl Transferase (HAT) enzyme. This enzyme transfers the acetyl group to the c-terminal end of the histone . As a result it will unwrap the DNA from histone .

  The process of wrapping the DNA to the histone by the help of Histone Deacetylease (HDAC) enzyme, is known as Deacetylation. When transcription is going on, after certain time there is no more requirement of protein. It is produced by the gene, the transcription process need to be blocked by the process of deacetylation.

- **Methylation -** The methylation process either helps in activation of genes or inhibition of genes. It depends on the situation or what kind of methylation is going on or where exactly the methylation going on. Depending upon that, it depends whether the gene will be accessible or the gene will be turned off. In some cases, if the methylation takes place in L3 residues of H3 in that case they will activate or unwrap the DNA from the histone and the gene will be activated and accessible. It will be transcribe it to produce the protein. When it takes place in L9 of H3 that turns it into an inactivated form and in that case, the DNA will wrap tightly in to the histones and the genes are not accessible.

METHODOLOGY ADOPTED TO DETERMINE EPIGENETIC VARIATION IN HUMAN DISEASE:-

- **DNA Methylation Analysis** – The DNA methylation refers to the addition of a methyl group (-CH3) to the base cytosine (C) linked by a phosphate bond to the base guanine (G) in the DNA nucleotide sequence . Both methylated and unmethylated CpG dinucleotides are present in the human genome. Mostly they are methyalated and the unmethyalated CpGs are not randomly distributed but they are clustered together in‘CpG islands ‘ are the promoter region of many genes. Methylation of CpG islands in cancer cells refers to silencing of gene expression.

Mechanism of action -

The gene promoter should be accessible to transcription factors and other regulatory units (e.g. enhancers) for the transcription process of gene. While DNA methyalation can prevent the binding of the transcription factors directly, it leads to change in chromatin structure that restricts access of transcription factors to the gene promoter.

Histones are the key protein component of chromatin which acts as reels for the wrapping of DNA that can be altered. For example, the methylated CpGs attract methyl-CpG-binding domain protein to

make 'repressor complexes' which results into histone modification and this leads to a more condensed chromatin structure (heterochromatin) as opposed to an open and active chromatin structure (euchromatin) required for transcription.

Maintenance and resetting of methylation marks – DNA methyalation which initiate the epigenetic marking of human genome is heritable as it transfers from one cell to another cell during cell division. It is also stable and allows a form of epigenetic 'memory'. DNA methylation process catalyses by the help of DNA methyltransferases. DNMT1 maintains the process. It allows control of developmental gene expression in specific tissues at specific times of embryonic development and takes place in maintaining of correct marking of imprinted genes.

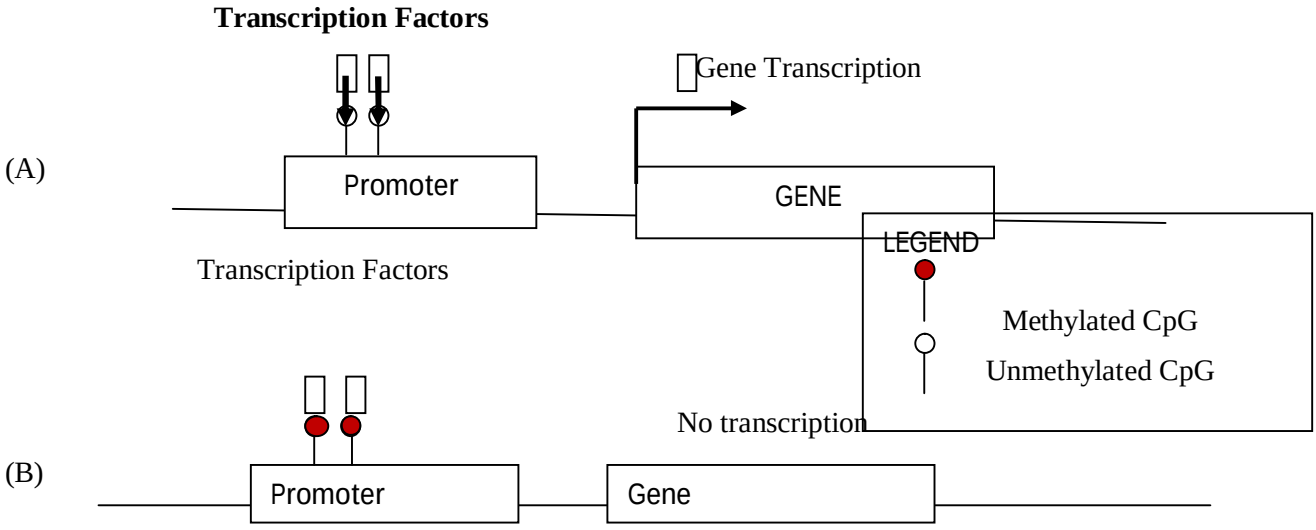

Figure 2:- Schematic representation of DNA methylation regulates gene expression. (A) The CpG island promoter is unmethylated and allows binding of transcription factors, which is required for transcription initiation. (B) The CpG island promoter methylation prevents binding of transcription factors and results in gene silencing [Adopted from Reference 5 with slight conceptual modification].

- **Histone Modification Analysis -** Histones are small basic protein which associates with the DNA in the eukaryotic nucleus to form chromatin. The four core histones (H2A, H2B, H3 and H4) can show sustainable modification of 20-40 N-terminal amino acids. H3 and H4 are very much fit for the modification because they mostly contain Lys , Arg residues . Histone modification is required for turning certain gene on and off inside eukaryotic genome because the idea of all the chemical modification (part of epigenetics) relate with the gene expression and genetic regulation of eukaryotic genome. In this point, we review the techniques that have been used to decode these complex histone modification patterns.

Posttranslational modification (PTM) of proteins play a key role in regulating the biological function of many polypeptides and the analysis of the modification status was done by using either a specialized gel system or a radioactive precursor molecule followed by complete protein hydrolysis and identification of the labeled amino acid[1]. The histone could be modified *in vivo* by acetylation, methylation or phosphorylation[3]. When histones can be purified in sufficient quantities and with a high purity, the modification occurs into the N-terminal end of the histone.

Mass spectrometry is the method of choice for analyzing PTM in histones [11]. The mass spectrometry methods currently used to map a modified residue are elaborate and require enrichment of the peptides that carry particular modifications. A variety of different methods are available to study complex histone modification patterns and these range from "bottom up approaches" to produce detailed and quantitative measurements of particular histone modification to "top-down-approaches" aimed at elucidating the interactions of different modification [7].

Table 1:- **: Methodology adopted in genome wide epigenetic analysis.(Adopted from Reference 10)**

Method of Analysis	**Techniques**	**Microarray Techniques**	**Next Generation sequencing**
DNA methylation analysis	Bisulfite reaction treatment	BiMP	BC-seq,WGSBS
	Concentration of methylated DNA by immunoprecipitation	MeDIP,mDIP,MIRA	MeDIP-Seq, MIRA-seq
	Application of methylation sensitive enzyme	HELP,MIAMI	HELP-seq
Histone modification analysis	Chromatin immunoprecipitation(ChIP)	ChIP-on-chip	ChIP-seq
MicroRNA analysis	Extraction of RNA	MicroRNA-chip	MicroRNA-seq
DNA replication timing analysis	Concentration of newly replicated DNA by immnunoprecipitation	Replication Timing-Chip	Replication Timing-seq

Abbreviation used-BC-seq-Bisulfite conversion followed by capture and sequencing,
BiMP-Bisulfite methylation profiling; ChIP-Chromatin immunoprecipitation;-chip-followed by microarray; HELP-HpaII tiny enrichment by ligation-mediated PCR; MeDIP-methylated DNA immunoprecipitation; MIAMI-microarray based integrated analysis of methylation by isoschizomers; MIRA-methylated CpG island recovery assay; WGSBS-Whole Genome Shotgun Bisulfate Sequencing

- **Non-coding RNA analysis: micro RNA -** There is so many evidences are present which describes that small non coding RNAs such as micro RNAs and long non coding RNAs , such as linc RNA , can regulate gene expression miRNAs (mature) are very small molecules which bears problem for their quantification because it is less efficiently precipitated in ethanol . It is necessary to avoid resuspension in ethanol when using the standard Trizol protocol for RNA isolation. miRNA have been reported to have greater stability than mRNAs

in sample obtained from tissues[10]. Mature miRNAs lack common sequence features, such as poly-A-tail or 5' cap that can be used to drive selective purification. Standard real time PCR methods can be applied to miRNA precursors only as a consequence.

An additional for the specificity of miRNA detection arises from the close sequence similarity of miRNA of the same family (mature miRNA, and pre-miRNA) and of the target sequence. Currently, various methodologies have been adopted to detect miRNAs, like Northern Bolt analysis with radio labeled probes, microarray-based and PCR-based analysis, single molecule detection in liquid phase, in situ hybridization and throughput sequencing [8]. The International Human Epigenome Consortium (IHEM) recommended that the identity and abundance of all non coding RNA species in a cell type should be determined and suggested that this should be accomplished by RNA sequence by next generation DNA sequencing after isolation of larger or small RNA species [10].

EPIGENETICS OF HUMAN DISEASE:-

1. ***Cancer Epigenetics*** – DNA methylation plays an important role in cancer development and progression. It can be accessed from body fluid and applied to cancer diagnosis as well as prognosis of cancer. Histone modifications are frequently altered in human cancers and the development of a histone modifications signature may be developed that will aid in the prognosis and treatment of cancers. These histone maps may also have potential in guiding therapy of human cancers and miRNAs are central to many cellular functions and they are disregulated frequently during oncogenesis. miRNAs expression profile may be more useful than gene expression profile which May be applicable to identifying various cancers or to stratify tumor in addition to serving prognostic or therapeutic roles [9]. Approaches are available for targeting enzymes such as the DNMTs , HATs, HDACs, HMTs, HDMTs. The development of drug-based inhibitors of these epigenetic modifying enzymes could be further improved through drug combinations or even natural plant-based products. Many of which have been found to harbor properties that can mimic the often more toxic and perhaps less bio-available epigenetic drugs that are currently in use. A flow chart of examples of three mechanisms leading to tumourogenesis is as follows –

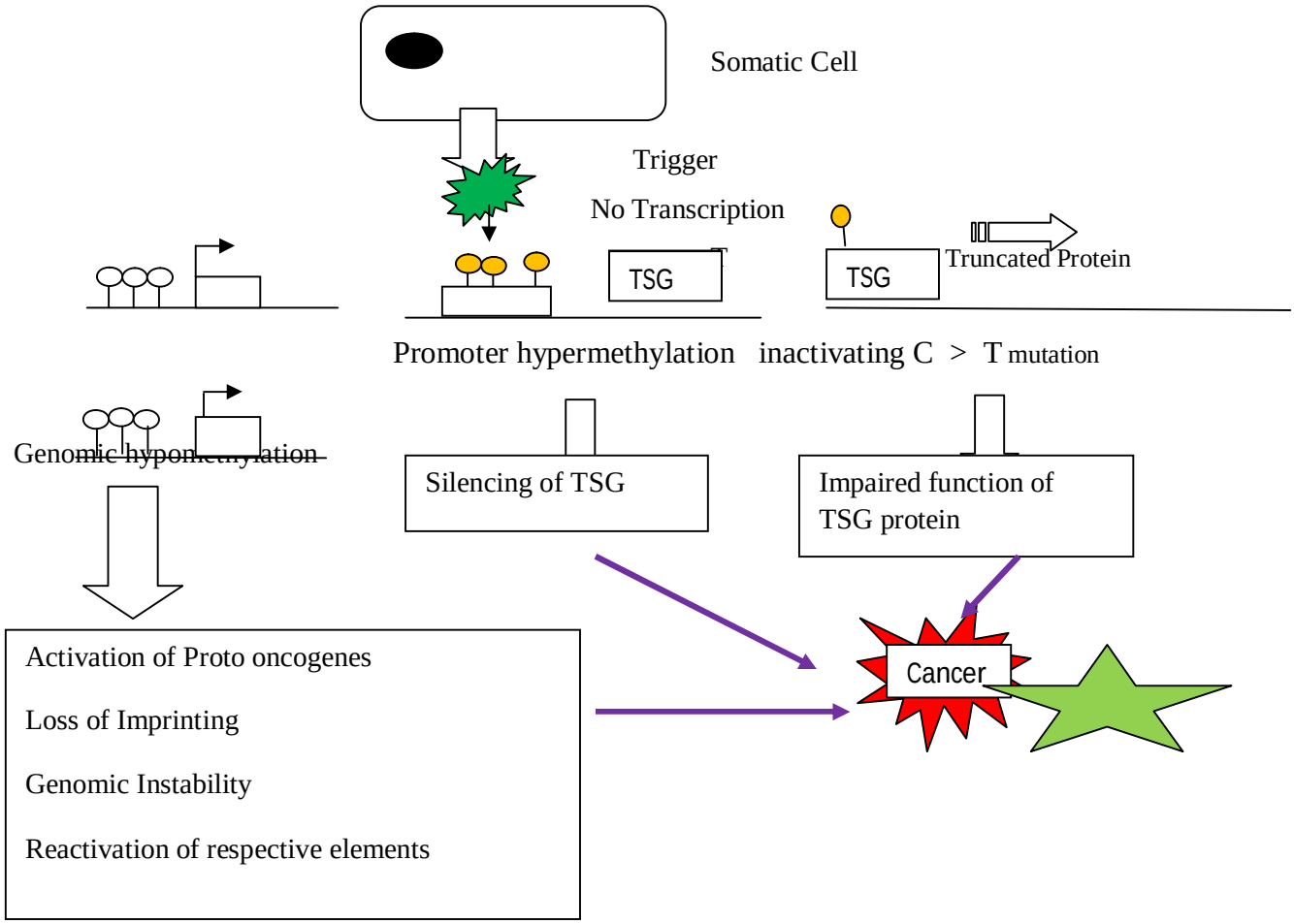

Fig. 3. Schematic representation of DNA methylation and cancer. Example of three mechanisms leading to tumourigenesis. (Abbreviation used- TSG-Tumour suppressor Gene) (adopted with slight conceptual modification from Reference 5)

2. Human Imprinting Disorders – DNA methylation and histone modifications can impact imprinting centers that control parent-of-origin-specific expression and lead to human imprinting disorders such as Prader-willi, Angelman, Silver-Russell and Beckwith-Wiedmann syndromes which involve epigenetic changes that contribute to these disorders and they manifest at a very young age [9]. Both epigenetic and genetic factors are often important in human imprinting disorders and the development of epigenetic therapy approaches in this particular area and the advantages are being made in understanding the epigenetic basis of human imprinting disorders which may provide breakthroughs in treating these tragic diseases.

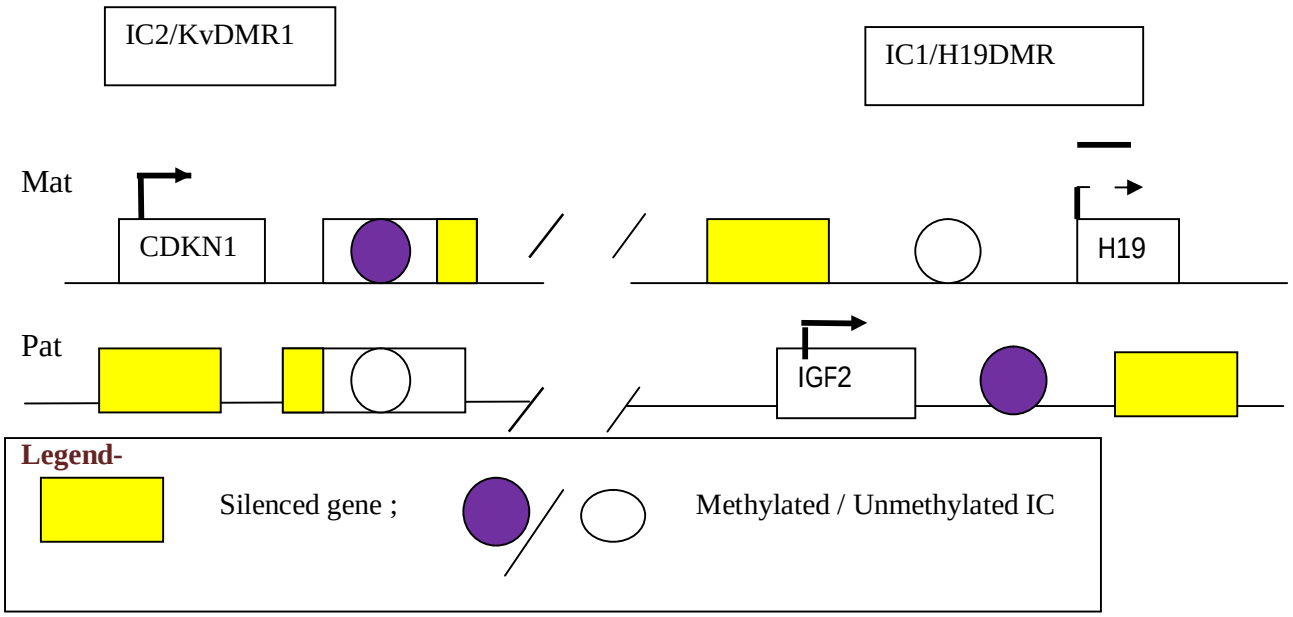

Fig.4. Schematic representation of Genomic imprinting of chromosome 11p15.5. Diagram showing the methylation status of the two imprinting Centres (IC1 and IC2) and genes that are selectively expressed from either parental allele. There is balance between the paternally expressed growth promoterIGF2 and the maternally expressed growth suppressor CDKN1C. Imprinting centre 1(IC1): the H19 DMR is methylated on the paternal allele, allowing expression ofIGF2 from the paternal chromosome. On the maternal allele (Mat), the H19 DMR is unmethylated, allowing expression of H19 from the maternal chromosome. Imprinting centre 2 (IC2): KvDMR1is methylated on the maternal allele, allowing the expression of CDKN1C from the maternal chromosome. On the paternal allele (Pat), KvDMR1 is unmethylated, resulting in silencing of CDKN1C. (Adopted from Reference 5)

- **Some other epigenetic diseases which now–a-days pays much attention are as follows-**
  - Neurological disease and epigentics.
  - Autoimmunity and epigentics.
  - Diabetes : the epigenetic connection.
  - Epigenetic and allergic disorders.
  - Epigenetics of obsesity.
  - Stemcell epigenetics in human disease

EPIGENETIC THERAPY - THE PHARMACOLOGICAL APPLICATION OF EPIGENETIC : Epigenetic therapy is potentially a very useful form of therapy which correct epigenetic defects by using some drugs and it is a new , rapidly developing area of pharmacology. When it is compared to genetic defects, it is thought to be more easily reversible with pharmacological intervention. However, epigenetic therapy has its limitations, such as the fact that

both DNMT as well as HDAC inhibitors may activate oncogenes due to lack of specificity which results into acceleration of tumor progression. Epigenetic states , once corrected , may revert to the original state because of the reversible nature of DNA methyalation patterns.

Most of the preclinical and clinical drug traits have involved various types of cancers such as solid tumors and haematological malignancies. Target for drugs in order to reverse epigenetic defects include enzymes such as HATs, HDACs, DNMTs and histone methyltransferases. Presently available epigenetic drug are classified into two groups depending upon whether they inhibit DNMTs or HDACs [6].

Table 2:- Classification of epigenetic drugs with therapeutic potential. (Courtesy-Reference 6)

DNMT inhibitors	**HDAC inhibitors**
(A) Nucleoside analogue inhibitors:-	**(A) Hydroxamates:-**
5-Azacytidine(5-aza-CR)	Trichostatin A
Decitabine(5-aza-cdR)	Suberoylanilide hydroxamine acid (SAHA)
Zebularine	**(B) Cyclic tetrapeptides:-**
(B) Non nucleoside analogue inhibitors:-	Depsipeptides
Procainamide	Apicidin
Procaine	**(C) Aliphatic Acids:-**
Epigallocatechin-3-gallate(EGCG)	Valproic acid
(C) Antisense oligonucleotides:-	Phenyl butyrate
DNMTI ASO	**(D) Benzamides:-**
	MS-275
	CI-994
	(E) Electrophilic ketones:-
	Trifluoromethyl Ketones
	α -ketoamides
Abbreviation used:-DNMT-DNA methytransferase; HDAC-Histone deacetyllase	

Table. 3 :- Classification of epigenetic drugs based on potentials therapeutic uses and developmental phase.(Courtesy- Reference 6]

Drug	Use	Developmental phase
(A) DNMT inhibitors:-	MDS	FDA approved for clinical use
→ 5 Azacytidine	Solid tumors	Phase II
	Leukaemia	Phase II
Decitabine	MDS	Phase II
	Leukaemia	Preclinical
Zebularine	Urinary bladder cancer	Preclinical
Procainamide	Prostate Cancer	Preclinical
Procaine	Breast Cancer	Preclinical
EGCG	Photocarcinogenesis	Preclinical
	Cancer of cervix	Preclinical
DNMTI ASO	Solid tumours	Phase I
(B) HDAC Inhibitors		
Trichoststin A	Breast cancer	Preclinical
	Ovarian Cancer	Preclinical
SAHA	Solid tumors	Phase I/II
	Leukaemias	Phase I/II
Depsipeptide	Leukaemias	Phase I/II
	Melanoma	Preclinical
	Colon cancer	Preclinical
Apicidin	Leukaemia	Preclinical
Valproic Acid	Bipolar disorder	In routine use(exact mechanism unclear)
	Breast and ovarian cancer	Preclinical
Phenyl butyrate	MDS; Laekaemia	Phase I
MS-275	Solid tumors	Phase I
CI-994	Solid tumors	Phase I
Trifluoromethyl ketones	Cancer	Preclinical
A ketoamides	Cancer	Preclinical
Abbreviation used:- MDS-Myelodysplastic syndrome; FDA-Food & Drug Administration; DNMT-DNA methyltransferase; EGCG-Epigallocalectin 3 gallate; ASO-Antisense oligonucleotide; SAHA-Suberoylanilide hydroxamine acid.		

EPIGENETIC DRUG COMBINATIONS RESULTING IN SYNERGISM –

Though DNA methylation and histone modification are known to interact, drugs that combines to inhibit DNMTs as well as HDACs may results in synergistic effects. In the cultured carcinoma cells, it was shown that four hyper ethylated genes could not be transcription ally reactivated with the HDAC inhibitor' trichostatin A' alone , but in the presence of low dose of DNMT inhibitor trichostatin A, decitabine resulted in robust expression of each of the four genes.

In addition to DNA methylation and histone modification, RNA mediated gene silencing is also a known epigenetic mechanism in gene expression, epigenetic drug combinations may need to target RNA as well.

FUTURE DIRECTION : This short review has covered the basic concept of epigenetic processes not only take many forms, but they also can readily become expressed as human diseases and also the processes like DNA methylation , histone modification , microRNA analysis and their

relevance to normal development and regulation of gene expression. Defects in this process can result in disorders affecting embryogenesis, imprinting disorders and cancer. Epigenetic is a rapidly developing area of human genetics and epigenetic therapy is a very new and rapidly developing area in pharmacology. Human genome has accelerated research into inherited diseases and cancer, it is anticipated that initiatives to define the normal human epigenome will enhance the progress toward better understanding of the role of epigenetics in human disease.

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