REVIEW

Epigenetic therapy for cancer

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Abstract: Epigenetics means the study of alterations in the genetic material that affect the phenotype but does not affect the genotype. Epigenetics cause alterations in cell properties, which are inherited; but it does not cause alterations in DNA sequence. Epigenetic mediated silencing of gene is of four types, which are DNA methylation, histone deacetylation, RNA associated silencing and Genomic imprinting. Other factors (environmental and xenobiotics) can also cause gene silencing but DNA methytlation and changes in histones of chromatin are two important changes, which are responsible for malignant diseases. Two groups of drugs are under development, which corrects the epigenetic alterations. These are histone deacetylation (HDAC) inhibitors and DNA methytransferase (DNMT) inhibitors. These drugs may be used in cancer because in cancer, hypermethylation of cancer suppressor gene causes gene silencing. Epigenetic therapy scope is likely to increase in future.

Keywords: Epigenetics, silencing, DNMTs, HDACs

INTRODUCTION

Inheritance of information on the base of expression of gene is called epigenetics. Genetics is opposite to it. So, the inheritance of information on the bases of sequences of genes is called genetics. Diploid human genome consists of approximately 6 billion DNA base pairs per cell packaged in 23 chromosomes. Human body consists of 50 trillion cells approximately, which forms 100 trillion meter of DNA per human. The distance of sun from earth is 150 billion meters. DNA is of such enough length that we can go from earth to sun and can again come back more than 300 times. Histones protein pack the larger chromosomal DNA into the microscopic space of eukaryotic nucleus (Hake *et al*., 2004). More compact DNA is formed by addition of H1 into it. Energy is provided by histones to fold the DNA. After this, chromatin becomes pack into very small volume. Histones are family of proteins having positive charge. These can bind with negatively charged DNA very tightly (Van Holde, 1988). Chromatin contains nucleosomes which are the basic units of chromatin. Nucleosomes consist of 166 DNA base pairs and nine histone proteins (Van Holde, 1988; Wolffe, 1999). In nucleosome H2A, H3, H4, H2B forms a discrete histone octamer which binds and rolled approximately 146 base pairs of DNA or about 1.7 turns of DNA. H1 protein again wraps 20 base pairs so that two full turns rolled around the octamer and having approximately 166 base pairs. CPG or CG sites are the regions where a guanine base follows cytosine. In CPG phosphate (P) joins both cytosine (C) and guanine (G). Promotors are the DNA regions where transcription of a particular gene is started. They are present in the vicinity of gene they transcribe. In the mammal's genome epigenetic changes takes place when cytosine comes before guanine. CPG dinucleotide may be methylated and non-methylated. 60-90% of dinucleotide is in methylated form while CPG island contain CPG dinucleotide in non-methylated form (Ng and Bird, 1999). In genome CPG dinucleotides are very occasionally found. They are not distributed uniformly. One in 80 dinucleotides is CPG dinucleotide. Genome contains 1% CPG content region. This region is known as CPG island (CPG clusters) (Bestor *et al*., 1988). CPG island is 90% in unmethylated form. CPG island are present in promoters region. In normal cells, unmethylated CPG island is present.

Active gene associates when CPG site is in unmethylated form, when methylation occurs at lysine 4 on histamine (H3K4), when there is histone acetylation and when chromatin is openly configurated. Active gene undergoes transcription. Inactive gene occurs when CPG regions are methylated, lysine 9 and 27 of histone H3 are methylated, histone is deacetylated with changed chromatin configuration. Four systems DNA methylation, Histone deacetylation, RNA associated silencing and genomic imprinting are responsible for epigenetic silencing initiation and maintenance. First three systems interact and stabilize one and other. The most well identified

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epigenetic alteration is CPG island hypermethylation. Disturbed pattern of DNA methylation which inhibited gene expression have been observed in cancers (Baylin and Herman, 2000; Das and Singal, 2004). Gene silencing may not be initially started by DNA methylation rather than prior silenced gene may cause CPG island methylation. It is also a fact that histone code alterations are required before cytosine methylation (clark and Melki, 2002). DNA hypomethylation can also causes cancer. Highly repeated DNA sequences show hypomethylation. Most commonly hypomethylation is observed in prostate cancer (Costello and Plass, 2001), Solid tumors (Kim *et al*., 1994) and hepatocellular tumors. DNA hypomethylation and hypermethylation are two independent processes (Ehrlich, 2002). Any disturbance in any of these systems inhibition of gene expression and silencing which ultimately causes epigenetic diseases such as cancer. Some other factors such as environmental and xenobiotics are also responsible for epigenetic silencing of tumor suppressive gene. Gene silencing inhibits gene transcription, which after transcription in normal cells regulates cell division, Suppress tumor and repair DNA. So in turn cancer develops. Cancer is uncontrolled growth of cells and proliferation, which can be prevented by modifying cellular machinery so that underlying genetic information are restored. Transcriptional inactivation is causally caused by DNA methylation (Bednarik *et al*., 1990). Many genes can be silenced in CPG island areas. These are p16, p15, Rb, p14, BRCA1, DAPK, MGM, TMSI etc (Das and singal, 2004). P16 methylation has been found in colon tumors.

DNA methylation

In epigenetic modifications DNA methylation is most widely studied in mammals. In promoter gene CPG dinucleotide is methylated. In DNA methylation there is transfer of methyl group by cytosine methyl transferase to C5 position of cytosine from S-adenosyl methionine (SAM) and DNA methyltransferases helps in this (Claus and Lubbert, 2003). This causes gene silencing which then inhibits expression of gene and its activation because its transcription is inhibited. These methylated regions are transferred heritably by cell division. In DNA methylation, Mecp2 (repressor) binds with methylated DNA then there is recruitment of complex containing histone deacetylase and a repressor of transcription. After this there is reduction in the affinity of gene sequence for transcriptional factors. This leads to inhibition of transcription of tumor suppressive gene. This gene regulates division of cell, suppression of cancer and cause repairing of DNA. Methyl CPG binding protein can also bind with cytosine in methylated form and this causes inhibition of binding of different factors which involve in transcription (Jain, 2003; Kass *et al*., 1997). When it binds, it causes attachment of histone deacetylases with methylated sites (Bird and Wolffe, 1999; Egger *et al*., 2004). Then some proteins and histones come in non-acetylated form (Bird and Wolffe, 1999; Claus and Lubbert, 2003). Methyl cytosine inhibits the binding of transcriptional factors to the methylated regions of DNA (Claus and Lubbert, 2003; Kass *et al*., 1997). Binding affinity may also be reduced (Jain, 2003; Mancini, 1998; Tate and Bird, 1993). Abnormal methylation can cause carcinogenesis. Epigenetic mechanisms are responsible for regulation of 600 genes. Alone DNA methylation may not be responsible for tumor. DNA methylation along with the hypermethylated gene may be the cause of gene silencing (Kouzarides, 2007). Methylation of cytosine is the most exclusive DNA Modification while histone changes are of many types.

DNA methyltransferases (DNMTs/DNA MTase)

DNA methytransferases are enzymes that catalyze the transfer of methyl group to DNA. All DNA methytransferases utilize a methyl donor, which is S-adenosylmethionine (SAM). DNMTs interact with proteins possessing transcriptional repression properties and histone deacetylases and directly cause inhibition of transcription (Bachman, 2001; Fuks *et al*., 2001). DNMTs cause gene silencing with or without DNA methylation (Barbara, 2003; Fuks *et al*., 2000; Robertson *et al*., 2000; Rountree *et al*., 2000). In mammals there are 3 active DNA methytransferases. These are DNMT1, DNMT3A and DNMT3B. DNMT2 is fourth enzyme, which is not DNA methytransferase. There are two kinds of methyltransferases denovo methylation methytransferases and maintenance methyltransferases. New cytosine methylation is done by de novo methyltransferases. They usually undergo expression during early embryonic life. Maintenance methytransferases methylate DNA when one strand in methylated already. They maintain methylation pattern established by de-novo methylation throughout an organism's life. DNMT1 is the DNMTase, which is most abundantly found in mammalian cells. In normal and cancer cells DNMT1 cause maintenance of pre-existing methylation. DNMT1 copies the methylated pattern in the newly formed DNA strand by acting on hemimethylated DNA. *In vitro* this enzyme is 7 to 100 times more active for hemi methylated DNA than for unmethylated DNA. Maintenance and de-novo methylation of tumor suppressor gene is carried out by DNMT1 in human cancer cells. DNMT1 contains 1620 amino acids. It contains regulatory and catalytic domain. Catalytic functions require both domains. De novo methylation is carried out by DNMT3 enzyme. DNMT3 is responsible for addition of methyl group to CPG sequence which forms hemi methylated then completely methylated CPG. So it performs methylation of unmethylated and hemi methylated CPG at the same rate. It has three members DNMT3A, DNMT3B, DNMT3L. CPG dinucleotide methylation by enzyme DNMT3A occurs at much slower rate than DNMT1 but it occurs at greater rate than DNMT3B. Structurally DNMT3L closely resembles with DNMT3A and DNMT3B. It is inactive on its own but for

DNA methylation it is critical. Sequence similarities of DNMT2 (TRDMT1) are same as that of 5-methycytosine methyltransferases. This enzyme is not responsible for DNA methylation but it methylates aspartic acid tRNA at position 38. Its name has been changed to TRDMT1 (tRNA aspartic acid methytransferase 1) to show its different functions.

Histone modifications

Histone alteration occurs in N-terminal area not in the body of histone because N-tail area is accessible. In active gene lysine 4 of histone H3 (H3K4) (Lachner *et al*., 2001) is methylated and in inactive gene lysine 9 and 27 of histone H3 are methylated (Jones *et al*., 1987-1991; Kouzarides, 2007). H3 hypoacetylation, methylation of H3K9 and phosphorylation are main transcriptional changes. These are responsible for changes in chromatin structure and silencing of gene. Histone acetylation is responsible for marking active transcribing regions. Histone deacetylated areas are inactive transcriptionally (Marushige, 1976). Histone methylation is responsible for marking both active and inactive areas (Baylin and Herman, 2000). DNA methylation can starts histones modification and on the other hand histone modification can also start DNA methylation (Jones and Baylin, 2002). At lysine residues histone acetyltransferases (HATs) are responsible for hypoacetylation of histone but histone deacetylase remove acetyl group and are responsible for histone deacetylation. This causes chromatin condensation, silencing of gene and inhibition of transcription. Chromatin condensation takes place when histone is deacetylated. Recruitment of histone decaetylases is caused by methyl CPG binding protein. HDACs cause histones and some other proteins deacetylation. This is responsible for silencing of gene, which develops cancer. DNMTs are also activated by HDACs. Methylation of lysine 27 and 9 on histone also causes gene silencing and cause transcription inhibition. Histone deacetylases are also recruited by DNMTs. It is also possible that histone mediated silencing of cancer suppressive gene is responsible for increased DNA methylation (Cortez and Jones, 2008). They both are responsible for gene silencing. Activation of gene is associated with histone acetylation and inactivation of gene is associated with histone decaetylation.

RNA associated silencing/Chromatin remodeling

RNA associated gene silencing is of two types transcriptional and post transcriptional. In post transcriptional gene silencing RNA forms heterochromatin and causes heritable transcriptional gene silencing. RNA transcriptional gene silencing occurs when RNA is present as anti-sense transcripts, non-coding RNAs and RNAi (RNA interference). RNA associated silencing also enhances DNA methylation and modifications in histone. In mammals RNAi associated silencing is not described (Panning and Jaenisch, 1998). However gene silencing by anti-sense RNA is reported. Anti-sense transcription is responsible for causing globin gene silencing and methylation of DNA in α-thalassaemia.

Genomic imprinting

It is specific allele silencing. In this one parental allele is silenced while other parental allele is not. It is maintained by methylated areas near or within the imprinted gene.

Other factors responsible for epigenetic variations

Environmental factors cause dys-regulation in DNA methylation pattern e.g. cigarette smoke and hormone. They develop cancer. Xenobiotics such as diethyl stilbesterol also change methylation pattern and are responsible for abnormal expression of gene. Nutritional factors also alter methylation pattern and can develop tumor.

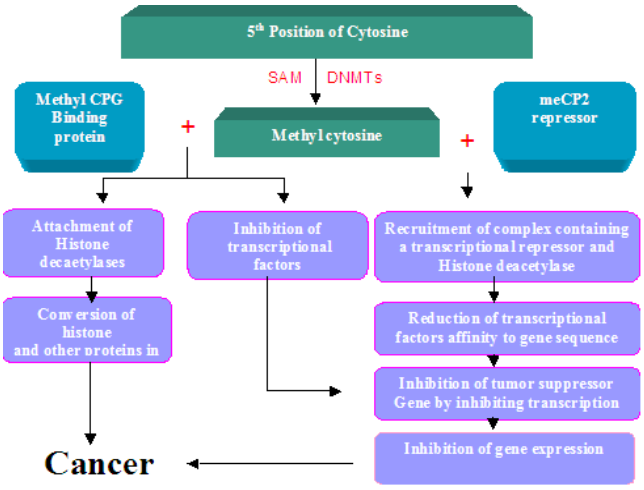

Fig. 1: Comprehensive diagram showing how DNA methylation causes cancer

DNA methylation role in prognosis and prevention

In clinical setting DNA methylation changes serve many functions. Hypermethylated gene occurs in cancer cells. While normal cells contain unmethylated CPG island. Keeping in view these, malignant changes can be assessed. These techniques can be used for detecting malignant alterations because hypremethylated gene precedes these changes. Altered methylation patterns can give prognostic information to clinicians. For example lung cancer can be detected by p16 gene hypermethylation. Methylation can be detected by various techniques.

Drugs used for epigenetic cancer therapy

The problem of resistance and tolerance to the existing drugs has created a decreased efficacy of these drugs in use. This problem has been tried to be overcome by increasing the drug delivery to the target site by the use of

polymers (Khalid *et al*., 2009; Hussain *et al*., 2011) or through nanotechnology (Naz *et al*., 2012; Ehsan *et al*., 2012), synthesis of new drugs, either by the use of proteomics (Qadir, 2011), or synthesis from lactic acid bacteria (Masood *et al*., 2011), or marine microorganisms (Javed *et al*., 2011). The modern era of research on medicine is searching for new drugs especially anti-inflammatory (Qadir, 2009), hypotensive (Qadir, 2010), hepatoprotective (Ahmad *et al*., 2012; Qadir *et al*., 2013; Mallhi *et al*., 2014; Qadir *et al*., 2014a; Qadir *et al*., 2014b; Saleem *et al*., 2014a), hypoglycaemic (Nisa *et al*., 2009; Qadir and Malik, 2010), amoebicidal (Asif and Qadir, 2011), anti-fertility, cytotoxic, antibiotic (Amin *et al*., 2012; Saleem *et al*., 2014b), spasmolytic, bronchodilator (Janbaz *et al*., 2013a), antioxidant (Janbaz *et al*., 2012), anti-diarrheal (Janbaz *et al*., 2013b) and anti-Parkinsonism drugs. Similarly, a large number of anticancer drugs are also under consideration for their positive effects (Ameen *et al*., 2012; Bokhari *et al*., 2012; Farooqi *et al*., 2013; Saleem *et al*., 2013; Iqbal *et al*., 2014).

Epigenetic alterations are reversible so these alterations can be therapeutically treated. We have synthetic drugs which inhibit enzymes e.g. histone decaetylases and DNA methyltransferases. Synthetic drugs are used in combination or as single agent in clinical setting. Combination therapy targets both mechanisms DNA methyaltion and histone modifications because of linkage between these two. Increase DNMT inhibitors doses cause cytotoxicity but lower doses can be given when they are combined with HDAC inhibitors. Another approach of combine therapy is that firstly use epigenetic therapy to cancer cells then use traditional chemotherapy, immunotherapy or interferon (Karpf and jones, 2002). HDACs alone are not responsible for gene hypermethylation unlike DNMTs. DNA methytransferase mostly are tested clinically. DNMTi and HDACi have been used for treating haematological cancers. They possess little efficacy for solid tumors (Hauschild *et al*., 2008). DNMTi are dominant over HDACi because HDACi alone cannot express the hyperacetylated gene (Herman and Baylin, 2003; Suzuki *et al*., 2002). Nutrition has been suggested to reverse abnormal epigenetic patterns. Dietary factors and epigenetic interventions decrease HDACs, MBD and DNMTs and these agents can change the DNA methylation status and restores histone in acetylated form. They can also increase methyl marks at promoters of inactive gene. After this, gene will be available for transcriptional factors, which activate transcription. DNA and modifications in histones are the common types of epigenetic alterations. Breast cancer treatment requires multidisciplinary therapies. Treatment options for treating breast cancer include options such as combination of surgery therapy, radiation therapy, cytotoxic chemotherapy and molecular targeted therapy. Recent treatment modifications now are focusing on the modifications, which are epigenetically controlled. These alterations depend upon the function of special enzymes histone deacetylases and DNA methytransferases. These enzymes are considered now a target for epigenetic treatment (Handel and Ebers, 2010). Inhibitors of these enzymes in epigenetic therapy show anticancer effects in malignant diseases.

Dna methyltransferase inhibitors

DNA methylation affects the gene expression in the gene regulatory regions. In mammalian genome a new DNA alteration which is 5-hydroxymethylcytosine causes demethylation of DNA (Tahiliani *et al*., 2009; Robertson *et al*., 2011). CPG island hypermethylation causes transcriptional gene inactivation. Hypermethylation often is found in promoters regions of gene. These regions involve in the regulation of cell cycle, repairing DNA and apoptosis. CPG island DNA hypermethylation have been observed in many malignancies, which include myelodysplastic syndrome. myelogenous leukemia (AML), (Voso *et al*., 2010; Hatziapostolou and Iliopoulos, 2011). DNA methylation is established and maintained by enzymes DNA methyltransferases (Bestor, 2000). In mammals active DNA methytransferases are DNMT1, DNMT3A and DNMT3B. In mammalian cell DNMT1 causes maintenance of methylation pattern of DNA. DNMT3A and DNMT3B performs denovo establishment of methylation pattern (Schaefer *et al*., 2009). Demethylation of DNA can be acquired by the failure of maintaining methylation after when DNA is replicated or by the processes which are independent on DNA replication involving base excision repair (BER) and nucleotide excision repair (NER) (Chen and Riggs *et al*., 2011; Gehring *et al*., 2009). TET1 an iron dependant α-ketoglutarate dioxygenase enzyme which is responsible for converting 5-methylcytosine to 5-hydroxy methyl cytosine and this causes demethylation. A recent drug development is mainly focusing on DNMT inhibitors, which include nucleoside, nonnucleoside analogues and antisense nucleotides. After inhibition of DNMTs gene could be reactivated which have been silenced in carcinogenic process by the DNA methylation and non carcinogenic state of cell can then be reconstituted. DNMTs inhibitors are not specific for any cancer type and they are used to treat many cancers (Cortez and Jones, 2006). Based upon their structure DNMTs are divided into three groups.

Nucleoside analogues

Nucleoside analogues cause inhibition of synthesis of DNA. They transform into nucleotide and then incorporate into the DNA. They forms covalent complexes with the enzyme DNMTs and cause their depletion and then finally methylation patterns reversed. Nucleoside analogues which are well characterized are of five types; 5-azacytidine, 5 aza-2-deoxycytidine (5-aza-cdr), 5-fluro-2-deoxycytidine, zebularine and decitabine.

Table 1: Classification of epigenetic drugs

DNMT inhibitors	HDAC inhibitors
Nucleoside analogues	*Short chain fatty acids*
5-azacytidine (5-aza-CR)	Butyrate (Phenyl butyrate)
5-aza-2'-deoxycytidine	Valproic Acid
5-aza-fluoro-2'-deoxycytidine	*Hydroxamates*
Zebularine	Trichostatin A
Decitabine (5-aza-cdR)	Suberoylanilide hydroxamic acid (SAHA)
Non-nucleoside analogues	ITF2357
Procainamide	LBH589
Procaine	Oxamflatin
Epigallocatechin-3-gallate (EGCG)	PCl-24781
RG 108	PXD101
Psammaplins	*Cyclic terapeptides*
NVP-LAQ 824	Apicidin
MG 98	Depsipeptide (FK228, Romidepsin)
Hydralazine	Trapoxin
Antisense oligonucleotides	Tasidotin
DNMT1 ASO	Cyclic hydroxamic acid containing peptide (CHAP)
	Benzamides
	MS-275 (Entinostat, MS-27-275)
	MGCD-0103
	CI-994
	Electroplilic ketones
	Trifluoromethyl ketones
	α- Ketoamides
	Miscellaneous
	Depudecin
	SNDX-275
	Isothiocyanates

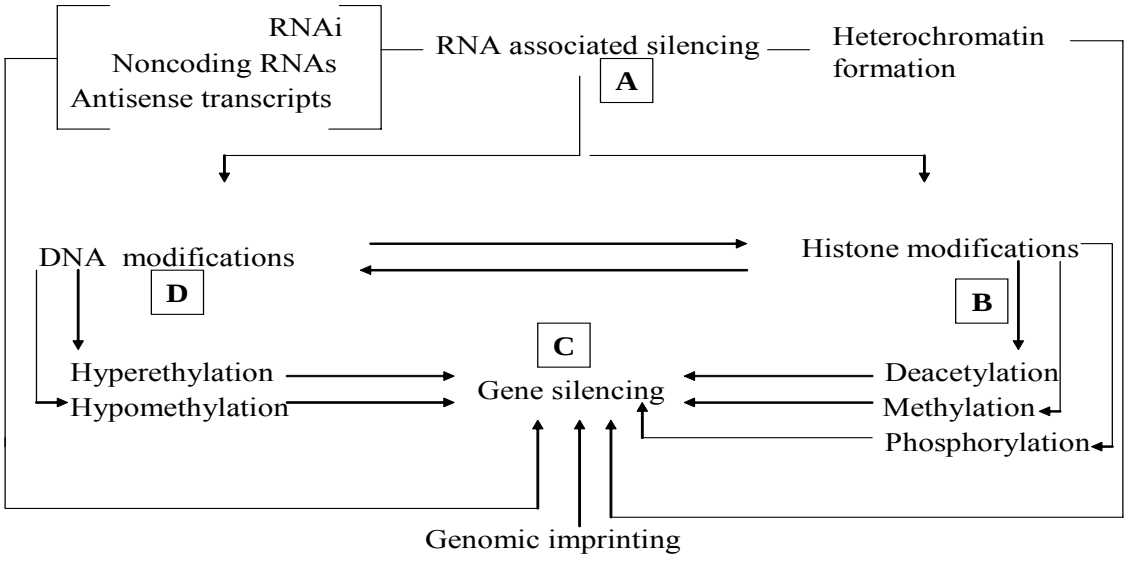

Fig. 2: (A) RNA associated silencing facilitates his tone modifications and DNA methylation. RNA associated silencing is transcriptional and post transcriptional. (B) His tone modifications can also start the process of DNA methylation. His tone modifications (acetylation and methylation, phosphorylation) cause gene silencing. (C) Genomic imprinting can also cause gene silencing. (D) DNA methylation initiates his tone modification. DNA hypermethylation and hypometylation are responsible for gene silencing.

5-azacytidine
5-azacytidine is a prototype DNMTi can be used for treating myelodysplastic syndrome. Its anticancer activity is due to two mechanisms, which are DNA methylation and cytotoxicity. It incorporated in both RNA and DNA. Mammalian cell treatment with 5-aza-CR causes defective rRNAs and tRNAs and inhibition of protein synthesis (Christman, 2002). This causes rearrangement in chromosomes and cytotoxicity (Kuo *et al*., 2007).

5-aza-2-deoxycytidine
It is cytosine analogue during replication becomes incorporated in the DNA. It inhibits both DNMT3 B and DNMT1. It causes in histone H3 and H4 acetylation at promoter regions. It activates silenced cancer suppressor gene. It increases the sensitivity of breast tumor cells to the anticancer agents (Mirza, 2010).

5-fluoro-2-deoxycytidine
This is DNA methyltransferase inhibitor. It inhibits methytransferase reaction (Beumer, 2008; Gowher and Jeltsch, 2004). This drug forms very toxic metabolites (Boothman *et al*., 1989).

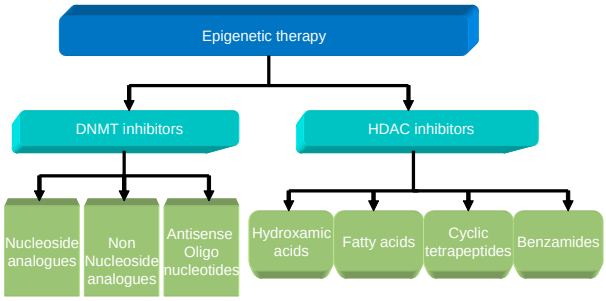

Fig. 3: Classification of drugs used in epigenetic therapy

Zebularine
It reactivates the gene which is silenced and inhibits methylation of DNA. It undergoes phosphorylation to diphosphate level then incorporates into the DNA. It causes post transcriptional DNMTs methyl CPG binding protein inhibition and causes modification in the histone acetylation state. Zebularine is less toxic than other DNMTi to breast tumor cell lines (Billam *et al*., 2010).

Decitabine
It is also a prototype DNA methytransferase inhibitor.

Non-Nucleoside analogues
Non nucleoside analogue which inhibit DNA methylation are very few. They do not incorporate into the DNA. They bind to the DNMTs catalytic region directly.

RG 108
It inhibits DNMTs in human cell lines *in vitro*. It reactivates cancer suppressor gene and causes demethylation.

EGCG
EGCG is a polyphenol compound present in green tea. Micromolar EGCG concentration increases the tumor suppressor gene transcription and decreases the DNA methylation (Fang *et al*., 2003). It will be tested in phase 11 and 111 clinical trials in future (Moyers and Kumar, 2004)

Psammaplins
They inhibit both HDACs and DNMTs (Pina *et al*., 2003).

NVP-LAQ824
It is derivative of psammplin. It has shown anticancer activity (Atadja, 2004). It is under phase 1 trial for hematologic malignancies.

MG98
MGAS inhibits DNMT1 mRNA translation. It gets hybridize with DNMT1 mRNA. MG 98 has low toxicity.

Hydralazine
Hydralazine cause transcriptional reactivation of tumor suppressor gene. Phase 1 clinical trials have shown that hydralazine is well tolerated and do not show common side effects of chemotherapeutic agents.

Antisense oligonucleotides
These are short nucleotide sequences which are complementary to Mrna. They hybridize and inactivate mRNA by inhibiting their translation.

Histone deacetylation inhibitors
In tumors, HDAC inhibitors are responsible for differentiation, apoptosis and growth arrest. The mechanism of histone deacetylation inhibitors is that they inhibit the enzyme histone deacetylase which in turn converts histone in acetylated form. By this different cellular processes which have become changed in cancer cells can then be restored to original state. Acetylated histone causes gene induction and upregulation that has silenced. HDAC inhibitors cause accumulation of hyperacetylated histones and they prevent cancer (Marsoni *et al*., 2011). LSD1 is first enzyme which demethylates histones. It was identified in 2004 (Shi *et al*., 2004). Histone deacetylation inhibitors are of four types: Short chain fatty acids, cyclic tetrapeptides, hydroxamic acids and benzamides (Fang and Ji, 2009). In the future HDAC inhibitors with chemotherapeutic agents may be used for treating breast cancer.

Short chain fatty acid
Butyrate
Butyrate was first histone deacetylation inhibitor which inhibited cell growth and induced apoptosis (Candido *et al*., 1978). Butyrate is responsible for hyperacetylation of H3 and H4.

Valproic acid
Valproic acid is an anti-epileptic drug with anticancer activity. It inhibits the enzyme histone deacetylase on estrogen sensitive and insensitive breast tumor cells. Valproic acid causes tumor cell differentiation.

Hydroxamic acids
Hydroxamates are effective at concentration of micromolar to subnanomolar.

Trichostatin A (TSA)
TSA was first hydroxamic acid. It most effectively changes the viability of breast tumor cells. It inhibits histone deacetylases and increases the histone acetylation so it may be used for the treatment of breast cancer (Kim *et al*., 2010). TSA combines with 5-aza-cdR and causes re-expression of silenced gene. It also possess anti-proliferative effects.

Suberoylanilide hydroxamic acid (SAHA)
It inhibit enzyme HDAC both class 1 and class 11. It possesses anticancer activity in hematological cancer. It is approved by FDA for treating cutaneous manifestation in T cell lymphoma patients (Steams and Zhou *et al*., 2007). SAHA phase 11 clinical trials are undergoing for breast and other tumors (Xu *et al*., 2005).

Cyclic Tetrapeptides
Trapoxin
Trapoxin small concentrations inhibits HDACs and is responsible for preventing acetylated histone conversion in deacetylated form. Trapoxin possess effect on cell differentiation.

Depsipeptide
Depsipeptide cause increase expression of p53 gene in breast tumor cells not in normal cells. It possess anticancer activity in cancer patients.

Tasidotin
Tasidotin clinical trials are ongoing for cancer treatment.

Cyclic hydroxamic acid containing peptide (CHAP)
CHAP inhibits enzyme HDAC *in vivo* and has effect on gene expression.

Apicidin
Apicidin has broad spectrum anti-proliferative effect in many cancers (Im *et al*., 2008). In breast cancer it inhibits the proliferation of cells by changing the regulatory protein expression and by induction of apoptosis.

Benzamides
MS-27-275
It is selective class 1 HDAC inhibitor. It has been investigated in phase 1 studies for cancer patients. Now its phase 11 trials are ongoing. Ms-275 causes cancer metastasis suppression (Srivastava *et al*., 2010).

CL-994 (N-acetyl-dinaline)
It is oral HDAC inhibitor. It has anticancer activity *in vitro* and *in vivo* (Riva *et al*., 2000). Its mechanism is not yet known. It is responsible for accumulating histone in acetylated form. It inhibits HDACs activity indirectly. It can be combined with other agents for use in cancer chemotherapy (Perabo and Muller, 2007).

CONCLUSION

Epigenetic trait results from chromosome alteration without changes in the gene sequence and it is a stable phenotype that can be inherited (Berger *et al*., 2009). Epigenetic mechanisms (DNA methylation and modification in histones) cause gene silencing without altering gene sequence. These events are heritable and reversible. The knowledge of epigenetic mechanisms offers opportunities for new drug discovery and therapeutic interventions**.** Epigenetic therapy (DNMT inhibitors, HDAC inhibitors) has a potential for treating cancer. It reverses the alterations in histone acetylation and DNA methylation pattern. DNMT inhibitors and HDAC inhibitors show synergistic effect. Epigenetic drugs are responsible for causing tumor suppressor gene reactivation. So the normal functioning of the cell can be restored. These drugs can be used alone or with the other agents having synergistic effects. It has been observed that conventional therapy combines with epigenetic therapy to produce optimal effects (Kristensen and Nielsen, 2009).

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