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Role of HDACs and DNMTs in cancer therapy: A review

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ABSTRACT: Although cancer has been considered as untreatable till now, but development of several biological agents have improved the survival and quality of life in some patients. A class of agents targeting epigenetic modifier enzymes have emerged with huge potential in cancer therapy. Novel compounds endowed with a histone deacetylase (HDAC) inhibitory activity and DNMT inhibitory activity are attractive therapeutic approach in recent time. Clinically, it is much simple to inhibit an enzyme than to induce one and this has directed the recent research to a new class of enzymes i.e. DNMT and HDAC. As a result of the earlier work it has become clear that HDACs are unique player in chromatin architecture, thus affect protein expression by altering the accessibility of Dna to the transcriptional machinery, which in turn affect transcriptional control and also activation of many tumour suppressor genes. Instead of it DNMTs are associated with hypermethylation of promoter and decrease its accessibility for transcriptional machinery. The extensive outcome of research in terms of structure, class, size etc. offers opportunities for the development of HDACi and DNMTi with improved specificity. In recent years, an increasing number of structurally diverse inhibitors have been identified that inhibit proliferation and induce differentiation and/or apoptosis of tumor cells both *in-vitro* and *in-vivo.* The question, that how epigenetic modifications associated with DNA or histones increases or decreases the accessibility of DNA to various factors that are involved in the reading of the DNA create interest in the researchers and lead to extensive research in this field.

Keywords: HDAC; HDACi; DNMT; DNMTi; DNA.

INTRODUCTION

Cancer is the outcome of genetic defects in general associated with the loss of tumour-suppressor genes function and/or hyperactivation of oncogenes [1]. Alterations in chromatin and dynamic changes in the nucleosomal packaging of DNA along with DNA methylation play a major role in controlling gene expression, cell division, survival and differentiation. Chromatin modifications play critical role in many diseases including cancer. During the last decades, evolutions of researches have shown that epigenetic alterations are involved in the repression of tumor suppressor genes and promotion of tumorigenesis [2]. DNA methylation, histone modifications and RNA-associated silencing are among the major epigenetic phenomena which work in relation to each other to perform epigenetic silencing[3].Egger *et al*., 2004). Acetylated histones remains linked with transcriptionally active chromatin and deacetylated histones with inactive chromatin [8]. Under normal conditions, chromatin acetylation is the result of balanced action between the histone acetyltransferases (HATs) and histone deacetylases (HDACs). Histone acetyltransferases (HATs) transfer acetyl groups from acetyl coenzyme A (acetyl-CoA) onto the ε-amino groups of conserved lysine residues within the core histones [9].

LITERATURE REVIEW

1. Dynamics of HATs and HDACs: Based on sequence homology Histones deacetylases are classified mainly into three major classes which includes class I ,class II, class III. Class I, includes HDAC1, 2, 3 and 8 are related to yeast RPD3 gene and have molecular weights of 22-55 kDa. Class II, includes HDAC

4, 5, 6, 7, 9 and 10 which are related to yeast Hda1 gene and are larger molecules with molecular weights varies from 120-135 kDa. Class III, also known as the sirtuins are related to the Sir2 gene and include SIRT1-7, and HDAC 11 has features of both Class I and II [10]. Class II HDACs are dynamic identity and keep on stirring between the nucleus and cytoplasm in response to cellular signals, where as Class I HDACs be a feature of nucleus and almost exclusively exist in nucleus [12][13]. Class I HDACs are widely expressed, as compare to class II HDACs which show varying degrees of tissue specificity. The class III HDACs, Sir2 usually deacetylates p53, inhibiting p53-mediated transcriptional activation and apoptosis [14]. HDAC6 is a feature of cytoplasm and function as regulator of cytoskeleton, cell migration and cell–cell interactions [15].

Dynamic changes along the histones in terms of acetylation or deactylation may affects transcription by two major pathways. Histone acetylation may alter the folding properties of the chromatin fiber which generate difference in the accessibility of DNA through structural changes [16][17]. Secondly, acetylations of lysine residues at specific sites affect binding surfaces for the recruitment of repressors and common chromatin regions some of examples includes AML, PML cases. In addition to aberrant recruitment of HDACs to specific loci, alteration in the expression of individual HDACs also found to be associated with the tumour samples for instance over-expression of HDAC1 observed in prostate18, gastric19, colon20 and breast21 carcinomas, while over expression of HDAC2 is observed in colorectal 20,22, cervical23 and gastric cancer24 . Increased expression of HDAC3 is seen in colon tumours20 and over-expression of HDAC6 was observed in breast cancer specimens [18][19][20][21][22][23][24]. Several cancer cell lines and human cancer tissues, including cancer of the stomach, esophagus, colon, prostate, breast, ovary, lung, pancreas and thyroid have shown that >75% of human cancer tissues and their corresponding non-cancerous epithelium have high expression of these class I HDACs [25]. Class 1 and class II HDACs have been observed in contrasting roles also, in apoptosis for instance histone deacetylase 1 and 2 differentially regulate apoptosis by opposing effects on extracellular signal-regulated kinase ½, over-expression of HDAC1 enhanced TGF-b1-induced apoptosis, and the rescue of HDAC1 expression in HDAC1 RNAi cells restored the apopto+++tic response of cells to TGF-b1. In contrast to it down regulation of HDAC2 by RNAi increased spontaneous apoptosis and markedly enhanced TGF-b1-induced apoptosis, suggesting that HDAC2 has a reciprocal role in controlling cell survival [26].

Some histone deacetylases (HDACs) interact directly with repressors and co-repressors of transcription as do HATs and activators, some of which are chromosome remodeling factors, and some of which are involved in cell cycle control. In AML1/ETO AML, recruitment of a multiprotein co-repressor complex containing N-CoR/Sin3/HDACs, causes histone deacetylation and gene transcriptional suppression [27][28]. In Acute promyelocytic leukemia (APL) a translocation t(15;17) found to be associated with disruption of RARα, at the molecular level, this translocation results in the fusion gene PML/RARA, encoding a chimeric protein with the ability to recruit the N-CoR/Sin3/HDAC transcriptional repressive complex to RA target genes[29]. Contiguous regions of gene suppression commonly occur through long-range epigenetic silencing (LRES) which is associated with regional histone deacetylation combined with subdomains of different epigenetic remodeling. In cancerous cells consolidation or effective reduction of the cancer genome commonly occurs in domains through a combination of LRES and LOH or genomic deletion, resulting in reduced transcriptional plasticity within these regions [30]. Histone acetylations play a role in regulation and transcription of genes controlling terminal B cell differentiation. Incubation of the L10A cells with the histone deacetylase (HDAC) inhibitors trichostatin A (TSA) and butyrate resulted in increased expression of *Blimp-1*, *J chain*, mad genes, surface CD43 Syndecan-1, decreased expression of *c-myc* and *BSAP/Pax-5* genes, decreased surface IgM [31]. In contrast to this in many cancer cells suppression of promoter is also prompt after histone deacetylase treatment. Aromatase involved in converting androgens to estrogens, HDACi LBH589 selectively suppresses human aromatase gene promoters, which are preferentially used in breast cancer tissue [32].

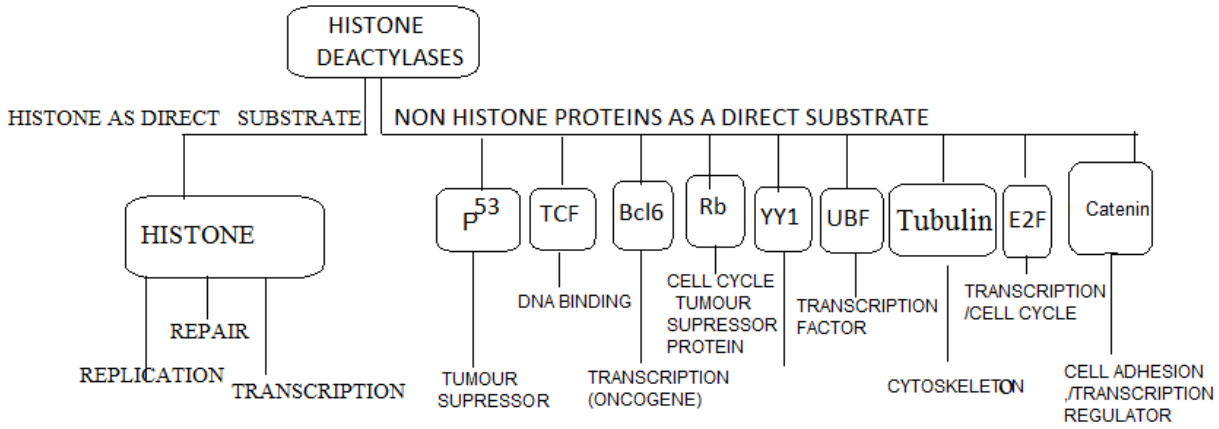

Figure 1. Showing various targets of histones.

2. HDAC inhibitors as anticancerous agents: Histone deacetylases are promising targets for cancer treatment. The major implication of HDACi in cancer therapy is contributed by activation of tumour suppressor genes, apoptotic pathways, cell cycle regulatory genes, resulted in arrest of growth, differentiation and apoptosis of many cultural transformed cells. *In vitro* a potent histone deacetylase inhibitor, FK228 (formerly FR901228) had shown more apoptotic and cytotoxic effects on human leukemia/ lymphoma cells and cell lines as compared to normal hematopoietic cells. Many of the histone deacetylase inhibitors observed till now have been found to induce p21, but were also differentially associated with tubulin acetylation, mitotic arrest, and cytotoxicity [33]. Anti-CD20 antibody rituximab is now essential for the treatment of CD20-positive B-cell lymphomas and HDAC inhibitors augment cytotoxic activity of rituximab by upregulating CD20 expression in lymphoma cells [33]. In a lymphoma cell line, Raji, a nanomolar concentration of FK228 induced G1 arrest and/or apoptotic cell death, depending on the concentration and exposure time [34]. Psammaplin A, a natural HDACi, induces cell cycle arrest and apoptosis in human endometrial cancer cells and significantly inhibits the proliferation of Ishikawa cells. PsA treatment resulted in H3 and H4 histone acetylations , up-regulated expression of cyclin-dependent kinase inhibitor, p21WAF1, and down-regulated expression of pRb, cyclins, and CDKs, which lead to induction of cell cycle arrest. Cell cycle analysis after PsA treatment concluded in increased proportion of cells in the G0/G1 and G2/M phases, and decreased ratio of cells in the S phase [35]. Hfw9 inhibitors for instance SAHA lead to down-regulation of class II HDACs in human cells. SAHA and MC1568 induces down-regulation of HDAC4 a class II HDAC, by increasing its specific sumoylation followed by activation of proteasomal pathways of degradation. HDAC 4 sumoylation mediates its repressive capacities whereas HDAC 4 degradation results into a transcriptional activation of target gene . An emerging concept from these findings suggested a cross link between acetylation, deacetylation and sumoylation pathways and class II specific HDAC inhibitors may affect different epigenetic pathways [36]. HDAC inhibitor LBH589 down-regulates DNMT 1 (DNMT1) expression in the nucleus of human breast cancer cells by hyperacetylation of Hsp90, which inhibits the association of DNMT1 with Hsp90 and lead ubiquitination of DNMT1 and ubiquitin-dependent proteasomal degradation of DNMT 1 [37]. OSU-HDAC42,a histone deacetylase inhibitor, blocks prostate tumor progression in the transgenic adenocarcinoma of the mouse prostate model [38]. Three dietary chemo preventive agents, butyrate, diallyl disulfide, and sulforaphane, also have HDAC inhibitory activity [39]. Phenylhexyl isothiocyanate causes Inhibition of HDAC activity in leukemia cells and lead to the increase in histone acetylation by the loss of repressive histone marks and causes the induction of p21 expression , cell growth arrest [40]. HDACi valproic acid inhibits cancer cell proliferation via down-regulation of the alzheimer amyloid precursor protein [41]. TSA a well known HDAC inhibitor can induce cell apoptosis in BGC-823 and SGC-7901 cell lines and this was also found to be associated with expression of acetylated histone H3[42]. A novel d-lactam-based HDACiKBH-A42 exerts an anti-tumor activity by inducing cell

cycle arrest and apoptosis in colon cancer both *in vitro* and *in vivo* and is a promising therapeutic candidate to treat human cancers[43]. Inhibition of HDAC3 produces mitotic defects independent of alterations in histone H3 lysine 9 acetylation and methylation [44]. A well known HDACi Sodium butyrate enhances the cytotoxic effect of antineoplastic drugs in human lymphoblastic T-cells [45]. With the evolution of researches, combination of HDACi with other 54 agents also raises new hopes in epigenetics therapy. Combined action of HDACi and a TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) receptor agonist had shown promisining results in preclinical settings [46]. Valproic acid enhances radio sensitivity of cancerous cells many times after exposure up to 24 h Irradiation, which has direct clinical application [47]. Liposomes loaded with histone deacetylase inhibitors for breast cancer therapy have been shown to be more effective against cancer cells [48]. TSA can sensitize HOS cells to the action of an antitumor agent genistein [49].
The HDACi LAQ824 induces human leukemia cell death through a process involving XIAP down-regulation, oxidative injury, and the acid sphingomyelinase- dependent generation of ceramide[50]. Vorinostat interferes with the signaling transduction pathway of T-cell receptor and synergizes with phosphoinositide-3 kinase inhibitors in cutaneous T-cell lymphoma [51]. HDACi had specific effects on cell fate decisions during myeloid development, they have been observed to modulates cell fate decisions during myeloid differentiation [52]. Novel HDACi chidamide has been observed to induce apoptosis in human colon cancer cells associated with increased acetylation levels of histone H3 and inhibition of PI3K/Akt, MAPK/Ras signaling pathways , resulted in G1 phase arrest of colon cancer cells and promoting apoptosis [53]. AN-999(pivaloyloxymethyl butyrate) has selective toxicity to acute leukaemia and drug-resistant primary leukemia and cancer cell lines [54]. Belinostat (PXD101) suppresses bladder cancer cell growth *in vitro* and *in vivo* [55]. Exposure (24–96 h) to butyrates of Kasumi-1 cells induced histone H4 acetylation results in morphological and immunophenotypic granulocytic maturation , Inhibition of proliferation and apoptosis via activation of caspase-9 [56]. Broad-spectrum histone deacetylase (HDAC) inhibitor PCI-24781, alone and combined with bortezomib induced concentration-dependent apoptosis that was associated with prominent G0/G1 arrest, decreased S-phase, increased p21 protein, and increased ROS in Hodgkin lymphoma and non-Hodgkin lymphoma cell lines[57].

3. Role in apoptosis: HDAC inhibitors have been observed to induce apoptosis in cancer cells that include both the intrinsic/mitochondrial and the extrinsic/death-receptor pathways. HDACi also affects the expression of genes involved in the extrinsic apoptotic pathways by up regulation of the proapoptotic and its associated genes and similarly by downregulation of antiapoptotic caspase inhibitors. Consistent with these effects, HDACi sensitize cancer cells to tumour necrosis factor alpha related apoptosis inducing ligand (TRAIL) lead to increased production and accumulation of reactive oxygen species [58]. In addition to it BH3-only proteins a major role in regulation and activation of the intrinsic apoptotic pathway. Further the activities of BH3-only depend upon the modification of Bid, Bad, Bim. Bid is cleaved and activated in response to different HDACi which results in activation of intrinsic apoptotic pathway [59].This study identified a new mechanism for activation of the 'intrinsic' apoptotic pathway by SAHA [60][61].
Bim get transcriptionally activated in response to HDACi treatment, for instance SAHA and TSA induce E2F1. Transcriptional up regulation of Bim and thereby promote apoptosis under conditions of high E2F1 activity [62]. HDACi selectively induces apoptosis in tumour cells. This ignites their therapeutic potential in cancer therapy.

4. Dynamics of DNA methylation: Till now three mammalian DNMTs, DNMT1, DNMT3A and DNMT3B, which have multiple domains to bind with co-repressors, have been described. DNMT2 has been supposed to work as tRNA methyltransferase [63][64]. DNMT3A/B act as *de novo* methytransferases and have been observed to play a important role in establishing DNA methylation patterns [65]. DNMT1 acts as a maintenance enzyme, maintaining patterns of DNA methylation and copies the pre-exiting methylation pattern to next generation following replication [66]. DNMT3B depletion resulted in reactivation of methylation-silenced gene expression, but did not induce global or juxtacentromeric

satellite demethylation as did specific depletion of DNMT1. DNMT3B has significant site selectivity that is distinct from DNMT1, regulates aberrant gene silencing, and is essential for cancer cell survival [67]. It was proposed that DNMT3B interacts with ZHX1 directly *in vivo* [68]. Three small regions in the amino-terminal one-third of the protein that are essential for DNMT1 function. Two of these regions (amino acids 124-160 and 341-368) border a large disordered region that regulates maintenance methylation activity. This organization of DNMT1's amino terminus suggests that the borders define the position of the disordered region within the DNMT1 protein, which in turn allows for its proper function [69]. DNMT3B and ZHX1 interact in vitro and *in vivo*, and are co-localized in the nucleus. Through this protein–protein interaction, transcriptional repression of DNMT3B is augmented, demonstrating a novel function of ZHX1 in the aspects of transcriptional regulation. DNA methylation has been considered as a stable component of the epigenome, established during development and fixed thereafter.

The blueprint of DNA methylation also varies throughout the cell cycle. Researchers concluded that during a single cell cycle, global levels of DNA methylation decreased in G1 and increase during S phase but little or less change in repetitive sequences throughout the cell cycle has been observed [70]. This decrease in DNA methylation is associated with Cell cycle-dependent accumulation of histones H3.3 and euchromatic histone modifications in pericentromeric heterochromatin [71]. DNA Methylation has a local effect on transcription and histone acetylation. Size of the methylated patch is not a key factor in transcriptional suppression alternatively it exerts utmost effect when it is in the transcription unit, and it is primarily a local effect. However, methylation outside of the transcription unit may potentiate the effect of methylation within the transcription unit. Direct links occur between histone acetylation and DNA methylation. Unmethylated DNA region are enriched with Acetylated histones and Acetylated histones are nearly absent from methylated DNA regions. It's a local effect and does not propagate along the DNA [72]. Silencing of neighboring genes linked with hypermethylation occurs independent of their euchromatic or heterochromatic location. In cancer, epigenetic modifications may target the promoter of individual genes, locally without preference for nuclear position and/or causing repositioning and has a important aspect in understanding characteristics of nuclear organization and gene expression patterns in cancer [73]. It was revealed that 174 CG-containing sequences were differentially methylated between G1 and S. Seventy-five percent of all the variations in DNA methylation detected in unique sequences represented hypomethylation at G0, with changes occurring in both CpG islands and non-CpG islands [74]. APC is a important regulator in Wnt-signaling pathway, the *APC* gene is involved in apoptosis and cell cycle arrest. In colorectal cancer cell lines, promoter methylation inhibits *APC* gene expression by causing changes in chromatin conformation and interfering with the binding of transcription factor CCAAT-binding factor [75]. DNMT1 is regulated in a complex fashion by E2F and other transcription factors through E2F-Rb-HDAC dependent and independent pathways. These findings suggest that DNMT1 is a target gene of these pathways in cell proliferation, cell transformation and tumor genesis [76]. It was shown that DNMT3B interacts with HDAC1, HDAC2, HP1 proteins, Suv39h1, and the ATP-dependent chromatin remodeling enzyme hSNF2H. These interactions connect DNMT3B to three other components of the epigenetic machinery and provide clear cut evidence about how DNA methylation patterns may be established within the chromatin environment [77]. Mammalian stanniocalcin-2 (STC2) has putative role in unfolded protein response and apoptosis STC2 expression was sporadically abrogated in human cancer cells due to CpG island promoter hypermethylation [78]. Epigenetic inactivation of the tumor suppressor gene RIZ1 resulted from promoter methylation and H3K9 modifications in hepatocellular carcinoma [79] . DNMT inhibitor 5- aza-2′-deoxycytidine (5-aza-dC), decreases level of DNA methylation, further leads to enhancement in transcription from centromeric and pericentromeric satellite repeats. Open conformations in pericentromeric in chromatin resulted in acetylation of histone H4, and di- and tri-methylation of lysine 4 on histone H3 [80]. Tobacco smoke is an important risk factor for various human cancers, including oesophageal cancer. How benzo [*a*]-pyrene diol epoxide (BPDE), a carcinogen present in tobacco smoke BPDE induced methylation of the RAR-β2 gene promoter and suppresses retinoic acid receptor-β2 expression by recruiting DNA (cytosine-5-)-methyltransferase 3A[81].

5. DNMT inhibitors as anticancerous agents: Recent researches has shown that DNA methylation is a major mechanism of epigenetic regulation that exerts its effects on transcription by avoiding the binding of specific transcription factors or by recruitment of methyl-binding proteins which in turn recruit additional chromatin modulators such as HDACs and HMTs leading to gene silencing. Any variation in DNA metylation pattern by mutation and/or by depletion of DNMTs or by inhibiting DNMTs can cause the various effect in cancers. A recent research has revealed that mutational inactivation of the *DNMT1* gene that potentially causes a genome-wide alteration of DNA methylation status may be a rare event during human carcinogenesis [82]. Depletion of DNMT 1 and/or DNMT 3b mediates growth arrest and apoptosis in lung and oesophageal cancer and malignant pleural mesothelioma cells [83]. DNMT3L is a novel marker and is essential for the growth of human embryonal carcinoma [84]. 5-Aza-CdR at limited concentrations induced inhibition of colorectal cancer Lovo cell proliferation as well as increased apoptosis caused by DNA damage, which was independent of the caspase pathway. Regarding the mechanisms, cytotoxicity against Lovo cells was experiential via down-regulation of DNMT 3a, DNMT3b and then reactivation of the RUNX3 gene [85]. Aza-nucleotides can become incorporated into DNA during replication and then are recognized by DNMT enzymes. A stable reaction intermediate is formed via the sulfhydryl side chain of the catalytic cysteine residue. Thus, DNMT is trapped and concomitantly degraded. 5-Aza-Deoxycytidine induces selective degradation of DNMT 1 by a proteasomal pathway that requires the KEN box, bromo-adjacent homology domain, and nuclear localization signal [86]. A quinoline-based compound, designated SGI-1027, inhibits the activity of DNMT1, DNMT3A, and DNMT3B and reactivates tumor suppressor genes P16, MLH1, and TIMP by blocking DNMT 1 activity and inducing its degradation [87]. Zebularine treatment inhibits cell growth in a dose and time dependent manner in MDA-MB-231 and MCF-7 cells followed by increased expression of p21, decreased expression of cyclin-D, and induction of S-phase arrest. At high doses zebularine induced changes in apoptotic proteins in a cell line specific manner manifested by alteration in caspase-3, Bax, Bcl2 and PARP cleavage [88]. Zebularine [1-(ß-D-ribofuranosyl)-1,2-dihydropyrimidin-2-one] acts as an inhibitor of DNA methylation and exhibits chemical stability and minimal cytotoxicity both *in vitro* and *in vivo* and it was further demonstrated that continuous zebularine treatment effectively sustains demethylation in human bladder cancer cells over 40 days [89]. DNA methylation inhibitor zebularine has been also observed to be effective against the development of marine T-cell lymphoma, supporting its implication in clinical trials [90]. Procaine is a DNA-demethylating agent that produces a 40% reduction in 5-methylcytosine DNA content in MCF-7 breast cancer cell lines and also restores the expression of RARβ2 gene; procaine also has growth-inhibitory effects in these cancer cells, causing mitotic arrest. Which further support the evidences that procaine is a promising candidate agent for future cancer therapies based on epigenetic [91]. Tea Polyphenol(-)-Epigallocatechin-3-Gallate inhibits DNMT and reactivates methylation-silenced genes in cancer cell lines human esophageal cancer KYSE 510 cells [92] and it has been supposed to inhibit DNMT by binding and blocking its active site , however generation of strong oxidising agent and oxidation of DNMT could be the another pathway of action [93][94].

5-azacytidine (5AC), Vidaza[95] 5-aza-2-deoxycytidin, Dacogen (DAC),Zebularine (ZEB) [96][97][98]. 1-(b-D-ribofuranosyl)-1,,2-dihydropyrimidin-2 one [99].,RG108[100]. ,Procaine [101]. Procainamide [102]., MG98 [103][110]. 5-Aza-2-Deoxycytidine delays androgen-independent disease and improves survival in the transgenic adenocarcinoma of the mouse prostate mouse model of prostate cancer [104]. Arsenic trioxide inhibits DNMT and restores methylation-silenced genes in human liver cancer cells [105]. Novel Oligoamine Analogues Inhibit Lysine-Specific Demethylase 1 and Induce Reexpression of Epigenetically Silenced Genes secreted frizzled-related proteins (*SFRP*) Human colorectal cancer cells [106]. 5-Aza-2V-deoxycytidine, as well as the HDACi trichostatin A, reactivates the growth-inhibiting genes TSP1, JUNB, and IGFBP3, which are suppressed in tumor-conditioned endothelial cells. DNMT inhibitors have angiostatic activity in addition to their inhibitory effects on tumor cells. This dual action of these compounds makes them promising anticancer therapeutic [107].

RG108 caused demethylation and reactivation of tumor suppressor genes with no affect on the methylation of centromeric satellite sequences these significant out comings establish RG108 as novel agent for cancer in epigenetic gene regulation[108]. Caffeic acid and chlorogenic acid inhibit the dna

methylation by the increased formation of S-adenosyl-L-homocysteine (SAH, a potent inhibitor of DNA methylation), resulting from the catechol-O-methyltransferase (COMT)-mediated O-methylation of these dietary Catechols which inhibit the methylation of the promoter region of the RARb gene [109]. Mithramycin A inhibits DNMT and metastasis potential of lung cancer cells [110]. Parthenolide, the principal bioactive sesquiterpene lactone alkylate Cys38 of p65 to inhibit nuclear factor-_B activation and exhibit anti-tumor activity in human malignancies, inhibits DNMT 1 (DNMT1) and reactivate tumor suppressor *HIN-1* gene in vitro , supposed to be associated with its promoter hypomethylation which established parthenolide as an effective DNA methylation inhibitor, representing a novel prototype for DNMT1 inhibitor discovery and development from natural structural-diversified sesquiterpene lactones [111]. Micro rnas has also opened the new era in the field of epigenetics. MicroRNA-29b induces global DNA hypomethylation and tumor suppressor gene *p15INK4b* and *ESR1 reactivation* via promoter DNA hypomethylation in acute myeloid leukemia by down-regulation of *DNMT3A* and *DNMT3B* [112].

6. DNMTs in apoptosis: Demethylating drugs causes apoptosis either by promoter demethylation of apoptosis effectors gene, signal,transducing mediators gene that involve in apoptotsis associated with both extrinsic or intrinsic pathways. In bladder carcinoma and B-cell Lines 5-AZA-CdR maintain the expression of DAPK1 which in turn have been observed to sensitized neoplastic cells to IFN- g triggered, TRAIL-induced apoptosis [113]. 5-AZA-CdR has been also found responsible for hypomethylation at caspase-8 promoter which in turn restores its expression in cancer cells and thus causes apoptosis [114][115]. EGCG inhibits growth and induces apoptosis in renal cell carcinoma through Tissue factor pathway inhibitor-2 TFPI-2 over-expression [116].

7. Combination Therapy: Various epigenetic phenomena linked to each other. The Methyl-CpG-binding Protein MeCP2 Links DNA Methylation to Histone Methylation as well as to histone deacetylation [117]. HDAC1, has the ability to bind DNMT1 and through a transcriptional repression domain in DNMT1 that functions, at least partly, by recruiting histone deacetylase activity [118]. Human methylation-dependent transcriptional regulator MBD1 bound to methylated DNA this binding causes a loop in MBD1 to fold into a major and novel DNA binding interface. Recognition of the methyl groups and CG sequence at the methylation site is due to five highly conserved residues that form a hydrophobic patch. The structure indicates how MBD may access nucleosomal DNA without encountering steric interference from core histones [119]. Promoter demethylation and histone acetylation mediate gene expression of MAGE-A1, -A2, -A3, and -A12 in human cancer cells,it was observed that that not only hypermethylation but also histone deacetylation is responsible for the mechanism underlying MAGE gene silencing [120]. In addition to it, combination of inhibitors also acts synergistically to cause re-expression of densely hypermethylated and transcriptionally silenced tumor suppressor genes in human cancer cells. Thus, reduction in DNMT and histone deacetylase activities that likely block epigenetically mediated gene silencing might provide a novel clinical strategy to help prevent the leading cause of cancer death in the United States for instance it has been observed that Inhibition of DNA Methylation and Histone Deacetylation Prevents Murine Lung Cancer [121]. The translocation t(8;21)(q22;q22) in acute myeloid leukemia (AML) results in the expression of the fusion protein RUNX1/ MTG8, which in turn recruits histone deacetylases (HDAC) to silence RUNX1 target genes [e.g. interleukin-3 (IL-3)]. Futher researches by using co-immunoprecipitation experiments has also shown that There is a physical association of RUNX1/MTG8 with DNMT1. These results suggest that RUNX1/MTG8 and DNMT1 were functionally interrelated [122]. Other examples include PML/RARA fusion protein recruits both DNMT and HDAC activities to block transcription of RA-target genes [123]. It was demonstrated that the hypermethylated genes *MLH1*, *TIMP3* (*TIMP- 3*), *CDKN2B* (*INK4B*, *p15*) and *CDKN2A* (*INK4*, *p16*) cannot be transcriptionally reactivated with TSA alone in tumour cells but the presence of low dose of 5-aza- 2´deoxycytidine (5Aza-dC), activation of genes takes place [124]. DNMT1 and HDAC are functionally interrelated support the combination of HDAC and DNMT inhibitors as a novel therapeutic approach. The cisplatin-resistant human ovarian cell line A2780/cp70(cell line) has the hMLH1 gene methylated and is resistant to cisplatin both in vitro and when grown as a xenograft in mice. Treatment of A2780/cp70 with decitabine

and belinostat results in a marked increase in expression of epigenetically silenced MLH1 and MAGE-A1 both in vitro and *in vivo* when compared with decitabine alone. Combination of decitabine and belinostat could have a role in the efficacy of chemotherapy in tumours that have acquired drug resistance due to DNA methylation and gene silencing [125]. Treatment of MCL cell lines with the DNMT inhibitor Decitabine resulted in reversal of aberrant hypermethylation and synergized with the HDAC inhibitor SAHA in induction of the hypermethylated genes and anti-MCL cytotoxicity [126]. Re-expression of methylation-induced tumor suppressor gene silencing is associated with the state of histone modification in gastric cancer cell lines [127]. By investigating various gastric and colon cancer cell lines Satoh A et al. demonstrated that aberrant DNA methylation and histone deacetylation of the 5' CpG island, but not the edge of the CpG island, appears to play a key role in silencing death-associated protein kinase expression in gastrointestinal malignancies [128]. Treatmaent with the known DNMT ,HDAC inhibitors has been also been found to be gene specific for instance in human colon cancer cell lines Colo-320 and SW1116, demethylation of the *CDKN2A* gene promoter in both cell lines induced by 5-aza-dC alone or in combination with TSA, the expression of both *CDKN2A* and *APC* genes increased. The treatment of TSA or sodium butyrate up-regulated the transcription of *p21WAF1* significantly by inducing the acetylation of histones H4 and H3, but change in transcription of *p53, p73, c-myc, c-Ki-ras* and *survivin* genes were observed [129]. A recent publication has revealed that Hydralazine showed no growth inhibitory effect on cervical, colon, breast, sarcoma, glioma, and head & neck cancer cell lines when used alone. On the contrary, valproic acid showed a strong growth inhibitory effect that is potentiated by hydralazine in some cell lines [130]. TSA may not only modify histone acetylation, but also potentially alter DNA methylation. TSA decreases DNMT3B mRNA stability and reduces its half-life from 4 to 2.5 hours. We established that protein synthesis is required for posttranscriptional regulation, suggesting the involvement of an RNase and/or key mRNA stabilization factor(s) controlling the DNMT3B mRNA stability Since the HDAC inhibitors are frequently used in epigenetic studies and are considered to be promising anticancer drugs [131]. DNMT Inhibition of 5-aza-2-deoxycytidine enhances apoptosis induced by histone deacetylase inhibitors depsipeptide and trichostatin A [132]. HDACs not only alone shown to play role in cancer treatment but in combination with other inhibitors they are found to be more effective for instance NPI-0052, a novel proteasome inhibitor, induces caspase-8 and ROS-dependent apoptosis alone and in combination with HDAC inhibitors in leukemia cells. Genistein or lycopene in breast cancer cells modulate the gene methylation and restore the expression of RARb2 gene and GSTP1 gene [133]. Selenium a mineral has shown to posses DNMT1 Inhibiting activity and affects the SAM/SAH and causes the hypomethylation of *p53* and *p16* gene [134].

CONCLUSIONS

Phase I Study of Vorinostat in Combination with Bortezomib for Relapsed and Refractory Multiple Myeloma. Although the potential reversal of epigenetic silencing of key genes holds promise as a novel treatment target, the potential role of hypomethylation in tumorigenesis remains controversial. Currently, much clinical interest is focused on combination with HDAC inhibitors, the compounds for which combination therapy has the greatest biologic rationale. Many of Agents with other therapeutic targets present additional opportunities to explore the combinations with DNMT inhibitors as well as with other active compounds. Such strategies may lead to improvement in response rates, remission, and survival while offering greater tolerability. The research continues and much more needs to be accomplished before these goals will be achieved.

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