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Review on Histone Deacetylase (HDAC) as a Potential Target for Cancer Chemotherapy

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Abstract

Cancer is currently the leading cause of mortality or disability worldwide. Current drug therapy is safe and effective and provides adequate control over Cancer. Despite good tolerability and reasonable safety profile of anticancer agents, delay for onset of action and side effects like Myelosuppression, Alopecia, Bone marrow toxicity remains barrier to effective therapy. These facts necessitate the search for the development of novel chemotherapeutic agents with better therapeutic profile. The effects of HDAC inhibitors on gene expression are highly selective, leading to transcriptional activation of certain genes such as the cyclin-dependent kinase inhibitor p21WAF1/CIP1 but repression of others. The functional significance of acetylation of non-Histone proteins and the precise mechanisms whereby HDAC inhibitors induce tumor cell growth arrest, differentiation and/or apoptosis are currently the focus of intensive research.

Keywords: Histone Deacetylase, Cancer, Target, Chemotherapy, HDAC inhibitor

Introduction

According to WHO cancer is the second principal cause of death globally. In 2008, an approximate 9.6 million deaths were because of cancer. Out of that approximately 70% deaths occurred in either low or middle income countries[1].

Cancer is a broad term to define. Abnormal cellular changes cause the uncontrolled growth and division of cells which results in cancer. Cancer is an abnormal growth, malignancy of cells. Out of more than 100 types of cancer some are included bellow.

Carcinoma is cancer in epithelial cells. Cancer of bone and soft tissues, muscle, fat or blood vessels is called Sarcoma. Cancer of lymphocytes and plasma cells called Lymphoma and Multiple myeloma respectively. Melanoma is cancer in melanocytes.

There are many targets such as DNA replication assembly, certain protein factors, enzymes etc to treat cancer. Still it is difficult to treat and cure cancer completely as medicines are not able to differentiate cancer cell and normal cells. This difficulty in identification of cancer cells and normal cells results into the medicines with side effects and adverse effect.

Here the selected target is Histone Deacetylase enzyme; present in the cell nucleus and also in cytosole.

Histone Deacetylase (HDAC) and Histone acetyl transferase (HAT)

In protein biosynthesis acetylation is one type of posttranslational modification. For Histone proteins deacetylation, is controlled by the Histone Deacetylase (HDAC) enzyme which is a zinc dependent metalloenzyme and the acetylation of Histone proteins by a Histone acetyl transferase (HAT) enzyme. Histone protein N-terminal lysine residue's deacetylation is HDAC enzyme catalysed. The positive charge on the N-terminal of Histone proteins increased through the deacetylation (Figure 1). The interaction between positively charged Histone protein and negatively charged DNA results in compact binding of Histone-DNA. The access of transcription factors get limited, and finally lead to transcriptional gene silencing. On the other hand, HAT causes acetylations of an amino group of lysine residues present on core Histone protein N-terminal (Figure 1). The acetylated neutral lysine doesn't have any interaction with negatively charged DNA. This results into Histone-DNA loose binding and gene-transcription activation due to the more relaxed chromatin state (Figure 2). Histone acetylation also linked with additional genome functions like chromatin assembly, recombination and repair of DNA2.

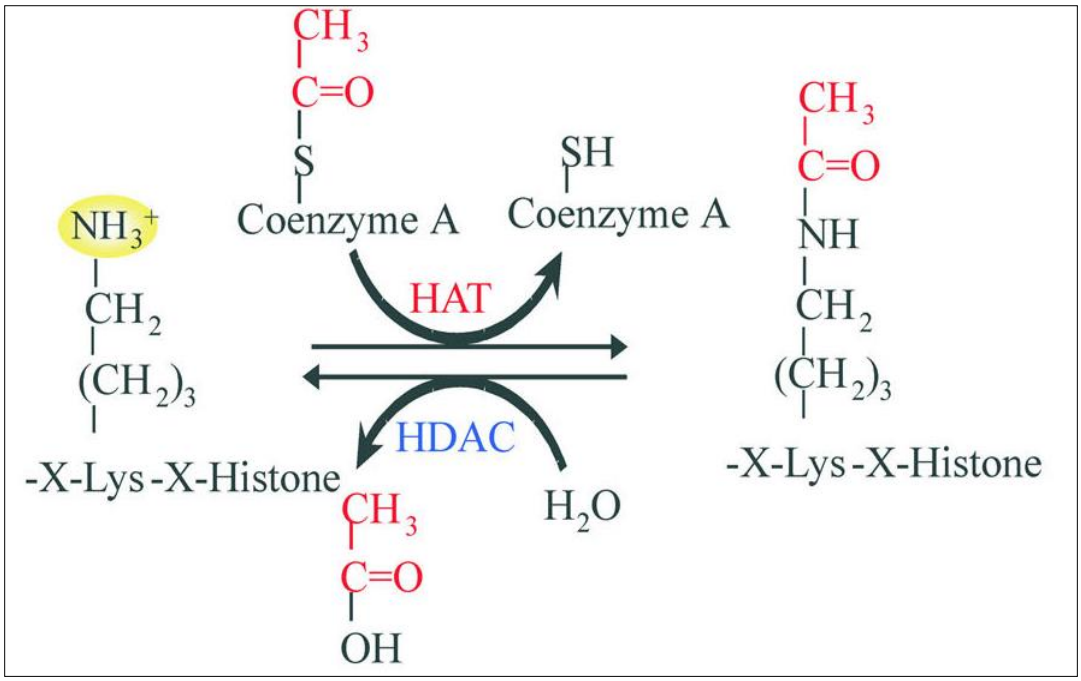

Figure No.1: Role of HAT and HDAC in transcriptional regulation: Histone modification by HAT and HDAC[2]

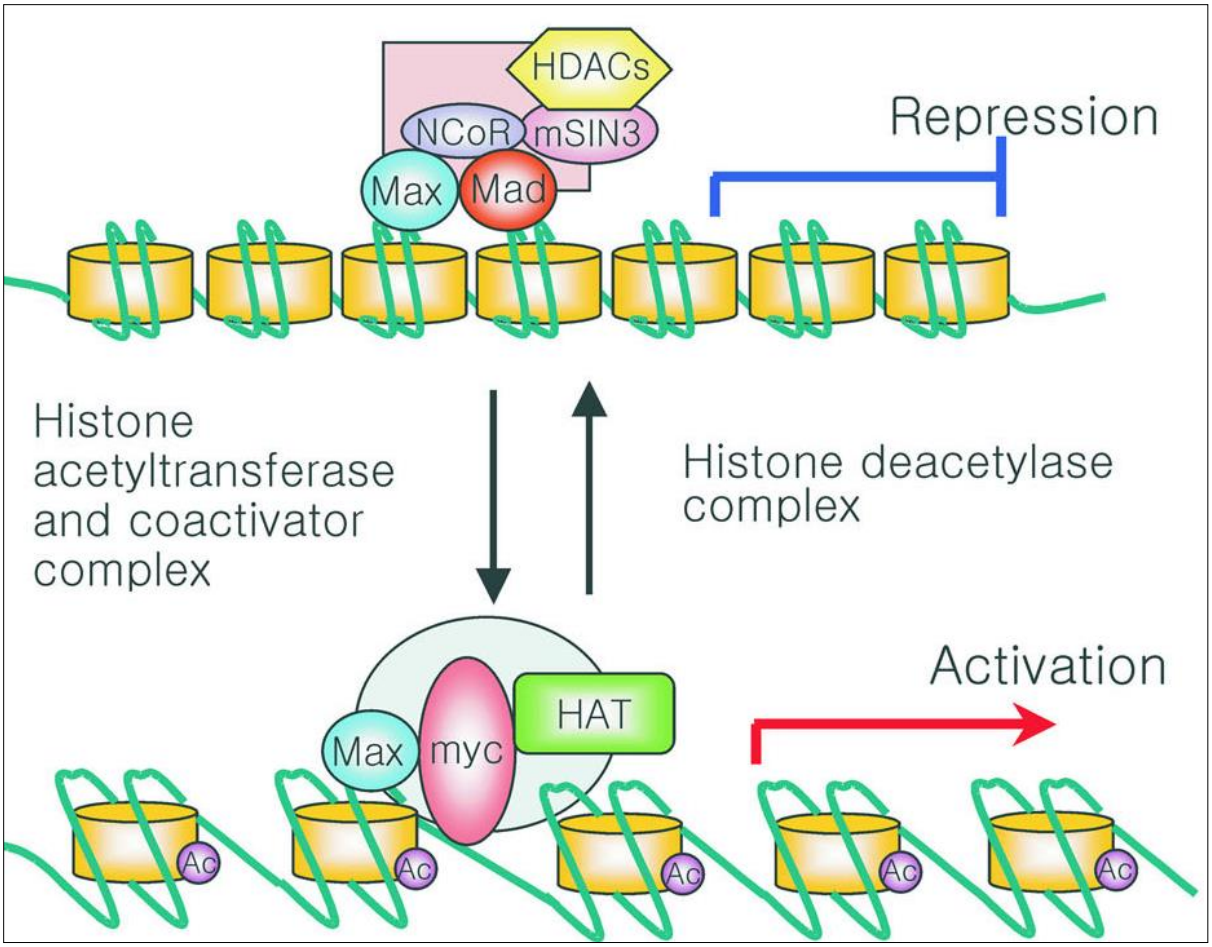

Figure No.2: Role of HAT and HDAC in transcriptional regulation: Regulation of gene-expression switch by co-activator or co-repressor complex[2]

Classification of Histone Deacetylase enzyme

The HDAC mainly sub classified in four main classes, Class I, Class II, Class III and Class IV (Table.No.1)

Table No.1: Classification of HDACs indicating location and cancer correlation

HDAC	Localization	Deregulation in cancer	Tumor	Amino acids
Class I				
HDAC1	Nucleus	Overexpression/underexpression	Esophageal, colon, prostate, CTCL	483
HDAC2	Nucleus	Overexpression/ mutation	Prostate, colon, gastric, endometrial, CTCL	488
HDAC3	Nucleus	Overexpression	Prostate, colon	423

HDAC8	Nucleus	Overexpression	Colon	377
Class IIa				
HDAC4	Nucleus/Cytoplasm	Overexpression/underexpression/ mutation	Prostate, colon, breast	1084
HDAC5	Nucleus/Cytoplasm	Underexpression	Colon, AML	1122
HDAC7	Nucleus/Cytoplasm	Overexpression	Colon	855
HDAC9	Nucleus/Cytoplasm	Overexpression/underexpression	Medulloblastomas, astrocytomas	1011
Class IIb				
HDAC6	Predominantly Cytoplasm	Overexpression	Breast AML, CTC	1215
HDAC10	Predominantly Cytoplasm	Overexpression	Heptocellular Carcinom	669
Class IV				
HDAC11	Nucleus/Cytoplasm	Overexpression	Breast	347

HDACs as drug target in cancer treatment: Rational behind it

Histone acetylation and deacetylation alters gene expression concerned with the genesis of cancer as well as in cancer, development and progression. This comprises the angiogenesis regulation that allows augmented growth of the tumor also the regulation of cell's adhesion, invasion and migration necessary for the metastasis. In the centre of a solid tumor hypoxia encountered which increases angiogenesis as well as increases HDAC expression, expression of HDAC1 induces the vascular endothelial growth factor (VEGF) the hypoxia responsive genes and hypoxia inducible factor - a (HIF-1a) but suppresses Von Hippel-Lindu (VHL) and tumor suppressor p53 all this results into the increase in angiogenesis[3]. On the other hand, HDAC inhibitors activate p53 and VHL and suppress VEGF and HIF-1a. Classe-I HDACs have an ability to control the extracellular matrix associated genes, which is extremely preserved from Caenorhabditis elegance to humans. HDAC1 suppresses a peptidase which results into suppression of tumor invasion called over expression of cystatine reduces cellular invasion[4]. The treatment by the low dose of HDAC ihibitors decreases v-Fos altered the fibrinoblast attack of HDAC inhibitor upregulates numerous genes like STAT6, protocadherin and RYBP[5]. Excessive expression of any gene out of all these genes reduce invasion but the function of HDAC activity is whether indirect or direct is still ambiguous. Class-I HDACs unswervingly controls E-cadherin gene expression which is vital for cellular adhesion. Lowering or failure of expression of E-cadherin gene leads to necessary first action in metastasis of epithelial cell invasion. The repressor Snail recruits the repressor mSin3A and HDAC1, HDAC2 for E-candherin promoter, suppressing expression of E-cadhering. Snail excessive expression in association with an

excessive hypo acetylated histone H4 and H3 at E-candherin promoter also augmented H3-K9 methylated histone and reduced H3-K4 methylated histone[6]. All such alterations are constant with suppressed gene expression after the treatment of prostate cancer cells by HDAC inhibitor VPA along with a peroxisome proliferator activated receptor-g (PPARg) agonist decreases the all-encompassing of these cells are concurrent with excessive E-candherin expression, mRNA and protein. HDAC3 as well as PPARg bind to the E-cadherin together and suppression in this case through the HDAC3. In the company of HDAC inhibitor and PPARg agonist togetherly, PPARg and HDAC3 are not remain bound to the promoter for the longer time, therefore H4 histone is hyper acetylated at the promoter region and expressed E-candherin. In acute lymphoblastic leukemia (ALL) HDAC inhibitor decreases chemokine receptor CXCR4 expression decreases the relocation so as to targets the ALL cells to the liver, spleen, brain and lymph nodes, all of this express excessive chemo attractant, the stromal cell derived factor[7]. Increased expression of intracellular adhesion molecules ICAM, tumor-derived endothelial cells by HDAC inhibitors is observed. In this way the binding tendency of lymphocytes to adhere to endothelial cells get enhanced which allows for improved tumor penetration by the lymphocytes. Less acetylated histone H3 and histone H3-K4 with methylation present in ICAM1 promoter isolated fromtumor derived endothelial cells. The treatment with methyltransferase inhibitor and HDAC inhibitors revere these modifications[8].

Transcription factors get transformed by acetylation status. Many properties of transcription factor, like their binding, active transcription, ability to DNA affected by deacetylation and acetylation. The tumor suppressor gene p53 expression gets affected by acetylation and deacetylation. The p53n activation can rapidly cause induces apoptosis or cell cycle arrest. The DNA binding ability of p53 increases with acetylation at K381, K370, K372, K373, K382 and K305 by p300/CBP as well as at K320 by PCAF and subsequently enhances its potential to stimulate target genes for transcription [(9,10,11)]. Acetylation at different sites on p53 takes place due to DNA damage causes unlike events. For example, DNA damage due to inhibition of topoisomerase enzyme induces acetylation at K373, which results into enhancement of apoptosis by activation of genes with Bax like low affinity p53 sites. Acetylation of K320 induced through the DNA damage due to alkylating agents which upregulates genes having high affinity p53 binding sites, as well as p21, and increases cell-cycle arrest such differential acetylation pattern can affect further phosphorylation of p53, which increases the nuclear localization of p53 with HDAC1 and SIRT1 stabilized by acetylation of K373[12]. Binding of SIRT1 to p53 and deacetylation at K382 reduces its activator function [(13, 14)]. The enhanced p53 mediated apoptosis observed in mice following DNA damage through inducing expression of proapoptic genes due to deacetylation of a K20 homolog [(15, 16)]. On p53 the acetylation through MYST family takes place selectively on K120, which is intended for apoptosis, but it's not meant for initiation of cell cycle arrest. In human cancer

cells K120R mutations have been observed, such cells carrying mutation at K120R apoptosis, as arginine cannot be acetylated p53 so not able to activate the genes Bax and PUMA. On the treatment with HDAC inhibitors depsipeptide of lung cancer cells, an increase in expression of p21 takes place, which is a result of the selective acetylation at K373 and K328 of p53 and recruitment of p300. In prostate cancer cells differential stabilization of the acetylation of K373 or K382 observed by HDAC inhibitor TSA or CG1521 respectively. Every separate acetylation action engaging jointly selective co-activator complexes and in such case to bring together the basic transcriptional assembly on the p21 promoter only the acetylation of K373 is sufficient[17]. Therefore it can be stated that treatment by diverse HDAC inhibitors of diverse cancers every one may have distinctive results (Table No. 2 and Table No. 3). The p53 stability gets modulated by acetylation or deacetylation. On p53 ubiquintylation takes place on lysine residue, but acetylated lysine is protected from ubiquintylation and consequent degradation with the proteasome. One example here, by Mdm2 E3 ubiquitin ligase through HDAC1 causes deacetylation at p53 enhances ubiquintylation plus degradation[18]. But current studies indicated that for p53 stability acetylation possibly will not be required. P53 half-life is normal though C terminal arginine replaced with lysine was in knock mice, while p53 activation following DNA damage differs[19]. The acetylation and deacetylation regulates the functions of the transcription factor family Runx. Runx1 is the vital regulator of ultimate hematopoiesis possibly act as an activator or repressor of transcription, p300 acetylates Runx1 at K24 and K43 the acetylated Runx1 function astranscription activator[20]. The transforming ability of Runx1 gets impaired through Mutations of lysine at which acetylation taking place. Runx3 is another Runx family member acts as gastric tumor suppressor and required for T-cell development. On binding of P300 to Runx3 acetylates at K148, K186 and K182 to it on stimulation of TGF-b. HDAC4/ HDAC5 reduce acetylation at higher extent and through HDAC1/ HDAC2 at smaller extent to Runx3. Ubiquitina tion of Runx3 through Smurf ubiquitin ligase get prevented by acetylation and increases its stability[21]. Ironically, based on Runx3 over expression in basal cell carcinomas, it can act as an oncogene. In this situation, still the consequence of acetylation remains unknown[22].

The binding ability of BCI6 to HDAC2 gets inhibited through acetylation at K376, K377 and K379 by p300, results into inhibition of BCL6 ability to repress transcription[23]. As HDAC inhibitors nicotinamide and TSA enhances BCL6 acetylation intensity indicate HDACs can deacetylate BCL6. The HDAC inhibitors TSA and nicotinamide causes cell cycle arrest as well as apoptosis in cells of β cells lymphoma. The transforming potential of BCL6 decresed with mutation of BCL6 that mimics acetylation PL2K is acetylated by p300 at K562, K565, K647, K650 and K653[24]. Acetylation increases the binding potential of PL2F to DNA with enhancement of transcriptional suppression of growth promoting genes. Mutation at the site of acetylation rigorously affects the potential of PL2E to repress cell growth. The B-cell

follicular lymphomas are characterized by the overexpress of the Bcl-2 antiapoptotic gene[25].

Table No. 2. Nonhistone proteins whose acetylation may be increased by HDAC inhibitors[26]

Sr No.	Protein	Intracellular Protein
1.	p53	Tumor suppressor
2.	c-Myb	Protooncogene regulates proliferation and differentiation
3.	GATA-1	Differentiation of blood cells
4.	Estrogen receptor-α	Stimulates growth of certain breast cancers
5.	TFIIE	General transcription factor
6.	Androgen receptor	Androgen-dependent transcription factor
7.	hsp90	Chaperone—targets proteins for degradation by proteasome
8.	α-tubulin	Microtubule component
9.	HMG-17	Unfolds higher order chromatin structure
10.	HMGI	Essential architectural component for enhancesome assembly
11.	TCF	↓ Transcriptional regulator
12.	PCNA	DNA repair and replication, cell cycle control, chromatin remodeling
13.	EKLF	Red cell–specific transcriptional activator
14.	ACTR	Nuclear receptor coactivator, HAT
15.	HNF-4	Transcriptional activation
16.	Importin-α	Nuclear import factor
17.	NF-κB	Regulates antiapoptotic responses
18.	ER81	Downstream effector of HER2/neu and Ras
19.	SF-1	Transcription factor expression of steroidogenic proteins
20.	Ku70	Suppresses apoptosis

21.	UBF	Structures DNA in ribosomal enhancesome
22.	Sp3	Transcriptional activator or repressor
23.	TAL1	Regulator of normal and leukemic hematopoiesis
24.	YY1	Multifunction transcription factor
25.	E2F1	Cell cycle activator required for progression
26.	MyoD	Stimulates cdk inhibitor p21

Table No 3. Tumor-associated proteins of which transcriptional expression is altered in response to HDAC inhibitor treatment of cells[26]

Sr No.	Regulated protein	Function (oncogenic or antioncogenic/tumor supressing)
	Down regulated by HDAC inhibitors (e.g., oncogenic)	
1.	HER2/neu	Growth factor receptor (EGFR class
2.	TGF-β	Regulates cell proliferation and differentiation through TGF-β type II receptor
3.	Thioredoxin	Disulfide reductase, cytokine activity, can inhibit apoptosis
4.	Telomerase	Prevents telomere erosion
5.	RECK	Regulates matrix metalloproteinases
6.	VEGF	Angiogenic factor
7.	bFGF	Angiogenic factor
8.	Myb/c-MyBL2	Oncogenic transcription factor–regulation of transformation and differentiation
9.	raf-1	Effector of Ras
10.	cyclin A	Cell cycle regulator
11.	cyclin B	Cell cycle regulator
12.	DAF	Complement inhibitor protein
13.	abl	Growth factor receptor, component of bcr/abl chimeric kinase
14.	DEK	Putative role in regulating chromatin structure and postsplicing events
15.	Proteasome	Degradation of misfolded or oxidized proteins
16.	Fas/Fas ligand	Proapoptotic

17.	Bcl2	Proapoptotic
18.	p53	Proapoptotic
19.	Bak, Bax, Bim	Proapoptotic
20.	c-myc	Inhibitor of differentiation
21.	Caspase 3	Cysteine protease involved in apoptosis, proapoptotic
22.	Carboxypeptidase A3 (CPA3)	Carboxypeptidase, putative role in regulating differentiation
23.	RECK	Negatively regulates matrix metalloproteinases
24.	p21WAF1/Cip1	Cell cycle regulation
25.	Gelsolin	Regulation of cell morphology
26.	ERα	Estrogen-activated nuclear receptor regulates transcription of estrogen responsive genes
27.	TSSC3	Regulates Fas-mediated apoptosis
28.	IGFBP-3	Augments IGF actions, promotes apoptosis, and inhibits cell growth
29.	TBP-2	Inhibits thiol-reducing activity of thioredoxin

Conclusion

The Histone proteins acetylation and deacetylation function in normal cells by HDAC and HAT respectively are well balanced. The impairment of this equilibrium of acetylation and deacetylation is often observed in cancers. Numerous in vitro studies have proved an effect of the inhibition of HDAC enzyme by its inhibitor on differentiation promotion, apoptosis and proliferation in many cancer cell lines. Additionally for HDAC inhibitors proposed therapeutic applications are use in neurodegenerative disease and inflammation. HDAC enzyme inhibition favor maximum histone to remain acetylated chromatin would not interact which increases asses to transcription proteins/factors. Therefore transcription of gene increases in such way induction of gene expression takis place which could be a common mechanism of HDAC inhibitors. The most commonly structurally HDAC inhibitors are analogues of hydroxamic acidc, cyclic peptides, benzamides, electrophilic ketones or short chain fatty acids. Mostly all contains three structural components, the catalytic zinc binding group to bind co-ordinately, a hydrophobic cap group to bind with active site entrance respectively and to separate these two a hydrophobic spacer group.

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