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• REVIEW •

Effects of histone acetylation and DNA methylation on p21^{WAF1} regulation

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Abstract

Cell cycle progression is regulated by interactions between cyclins and c yclin- dependent kinases (CDKs). p21^{WAF1} is one of the CIP/KIP family which inhib its CDKs activity. Increased expression of p21^{WAF1} may play an important role in the growth arrest induced in transformed cells. Although the stability of the p21^{WAF1} mRNA could be altered by different signals, cell differentiation and numerous influencing factors. However, recent studies suggest that two known mechanisms of epigenesis, i.e.gene inactivation by methylation in promoter region and changes to an inactive chromatin by histone deacetylation, seem to be the best candidate mechanisms for inactivation of p21^{WAF1}. To date, almost no coding region p21^{WAF1} mutations have been found in tumor cells, despi te extensive screening of hundreds of various tumors. Hypermethylation of the p2 1^{WAF1} promoter region may represent an alternative mechanism by which the p21$^{WAF1/CIP1}$ gene can be inactivated. The reduction of cellular DNMT prote in levels also induces a corresponding rapid increase in the cell cycle regulator p21^{WAF1} protein demonstrating a regulatory link between DNMT and p21 ^{WAF1} which is independent of methylation of DNA. Both histone hyperacetylation and hypoacetylation appear to be important in the carcinoma process, and induct ion of the p21^{WAF1} gene by histone hyperacetylation may be a mechanism by which dietary fiber prevents carcinogenesis. Here, we review the influence of hi stone acetylation and DNA methylation on p21^{WAF1} transcription, and affect ion of pathways or factors associated such as p53, E2A, Sp1 as well as sever al histone deacetylation inhibitors.

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INTRODUCTION

Cell cycle progression is regulated by interactions between cyclins and CDKs[1,2]. Especially, the transition of G_1 to S phase is known to be regulate d by a family of negative cell cycle regulators, CDKIs. The latter includes two families, the CIP/KIP family and the INK4 family[3-6]. p21^{WAF1} is one of the CIP/KIP family[7,8]. Increased expression of p21^{WAF1} may play a crucial role in the growth arrest induced in transformed cells[9].

p21^{WAF1} was first cloned and characterized as an important effector that a cted to inhibit cyclin-dependent kinase activity in p53 mediated cell cycle arrest induced by DNA damage[10,11]. It has been shown that this is a G C-rich region in the human p21^{WAF1} promoter[12]. Although the sta bility of the p21^{WAF1} mRNA could be altered by different signals cell diff erentiation[13] and oxidative stress[14] as well as numerous inf luencing factors including decorin[15], Ras/Raf protein[16], TGF-β[17] and Tax of human T cell leukemia virus type 1(HTLV-1)[18,19]. However, two known mechanisms of epigenetic modification, gene inactivat ion by methylation in promoter region and changes to an inactive chromatin by hi stone deacetylation, seem to be the best candidate mechanisms for the inactivati on of CIP/KIP family[20]. In this review, we focused on the methylation, histone acetylation and some transcription factor, co-transcription factor ass ociated with acetylation.

DNA METHYLATION AND HISTONE ACETYLATION

The post-translational modifications include acetylation, phosphorylation, meth ylation, ubiquitination and ADP-ribosylation[21]. In mammals, methylati on of the 5' position of cytosine in the CpG dinucleotide sequence is the only n aturally occurring covalent modification of the genome. The enzyme DNA 5-cytosi ne methyltransferase (DNMT) catalyzes the transfer of a methyl group from S -adenosylmethionine to the 5 position of cytosines residing in the dinucleotide sequence CpG[22]. DNA methylation patterns correlate inversely with gene expression[23] and, therefore, DNA methylation has been suggested to be an epigenetic determinant of gene expression.

DNA methylation is believed to be an on-off switch in gene expression, CpG isla nds present in the promoter regions have been shown to be susceptible to hyperme thylation in many cancer cells[24]. CpG islands near promoters and 5' re gulatory region are usually unmethylated in normal somatic cells. In contrast, widespread methylation of CpG islands occurs in autosomal genes and leads to the silencing of the genes during oncogenic transformation.

DNA in eukaryotes is packaged with histone and non-histone proteins into chroma tin. In general, regions of chromatin that are hyperacetylated are transcription ally active, whereas regions that are hypoacetylated are silenced. Indeed, a glo bal increase in core histone acetylation does not necessarily induce widespread transcription[25]. Histone acetylation results in charge neutralization and separation of DNA from the histones allowing nucleosomal DNA to become more accessible to transcription factors. Histone acetylation is believed to stabiliz e local nucleosomal structure, thereby allowing transcription factors and the ba sal trancriptional machinery access to DNA. Hyperacetylation of histones has bee n shown to mark open chromatin and to be required for trancriptional activation[26].

Histone acetylation is a reversible process: histone acetyltransferases (HATs) t ransfer the acetyl moiety from acetyl coenzyme A to the lysine neutralizes the positive charge, and histone deacetylases (HDACs) remove the acetyl groups re-es tablishing the positive charge in the histones. At least six human HDAC enzymes exist, and for higher eukaryotes, HDAC1 was first purified using an affinity mat rix based on the deacetylase inhibitor trapoxin[27]. HDAC inhibitor incl ude trichostatin A(TSA)[28,29], trapoxin (TPX)[30], Butyrate[31,32], MS-27-275 (a synthetic benzamide derivative)[33] and Apicidin[9,34]. Due to the inhibitory effects of the compounds of

endogeno us genes that plays significant roles in G1-S progression of the cell cycle, HD AC inhibitors have been considered to be a novel class of cancer treatment agent [34].

Methylation is not genomically uniform, as unmethylated CpG are found preferenti cally in transcriptionally active chromatin. The highest density of nonmethylated CpG islands, which usually contain promoter or other regulatory DNA that is required for active transcription of a gene. CpG island chromatin is enriched in hyperacetylated histones and deficient in linker histones[35]. Recent stu dies have suggested a strong link between histone acetylation, chromatin remodel ing, and gene regulation[26,36,37]. The results from many papers establi shed a link between DNA methylation, histone acetylation and sequence-specific DNA binding activity. In general, CpG island chromatin was found to contain highly acetylated histone H3 and H4. Deacetylation of histone H3 and H4 by the HDACs presumably leads to the formation of a chromatin environment that inhibits tran scription[38]. Hypoacetylated, transcriptionally silenced regions are of ten methylated[39], Furthermore, methylated DNA is transcriptionally rep ressed, but only under conditions in which the methylated template is assembled into uncleosomal structures[40], methylation density defines the level o f histone acetylation[41]. There are the roles of MeCP2, MBD1, MBD2, and MBD3[35], NuRD (nucleosome-remodeling histone deacetylase)[42,43] and DMAP1[44], as well as DNMT1[44,45] in the linkage of methylation with acetylation.

METHYLATION AND TRANSCRIPTION EXPRESSION OF p21^{WAF1} GENE

Usually, one could propose the negative regulation of p21^{WAF1} on the bindi ng of DNMT1 with PCNA in normal cells[46], however the loss of p21^{WAF1} from PCNA complexes could cause abnormal gains of methylation during repair of DNA damage[47]. Moreover, the p21^{WAF1} gene transcription level is regulated by methylation, due to that p21^{WAF1} promoter contains high d ensity of potentially methylatable CpG dinucleotides clustered around the initia tion site of transcription (Figure 1).

CpG island
-243
CGAGGGACTGGGGGAGGAGGGAAGTGCCCTCCTGCAGCA**CGCG**AGGTT**CCGG**GA**CCGG**CTGGCCTGCTGGA
ACT**CG**GCCAGGCTCAGCTGCTC**CGCG**CTGGGCAGCCAGGAGCCTGGGC CC**CG**GGGAGGGCGGTC**CCGG**G
CGG**CGCG**GTGGGC**CG**AG**CGCG**GGT**CG**CCTCCTTGAGGCGGGC**CCGG**G**CG**GGGCGGTTGTATATCAGGGC**CG**
CGCTGAGCTG**CG**CCAGCTGAGGTGTGAGCAGCT **G**
-1 |→+1

Figure 1 There are more CpG island at the domain near by the t ranscription start site in the promoter of p21^{WAF1} gene.

Dr. Nass *et al*[48] transfected three antisense DNMT1 (pCMV TMH) into human breast cancer MDA231 cell line, and found that the reduced DNMT1 protein and up-regulation of p21^{WAF1} suggesting that DNMT protein levels were inv ersely correlated with the level of p21^{WAF1} in breast cancer cells.

To date, almost no coding region p21^{WAF1} mutations have been found in tumor cells, despite extensive screening of hundreds of various tumors[49-51]. Hypermethylation of the p21^{WAF1} promoter region may represent an alter native mechanism by which the p21$^{WAF1/CIP1}$ gene can be inactivated. DNMT and p21^{WAF1} compete for the same binding site on PCNA, an increase in DNMT expression might promote dissociation of p21^{WAF1} from PCNA, perhaps making p21^{WAF1} more susceptible to ubiquitination and proteasome degradation [52]. A decrease in DNMT expression would then be expected to have an opposi te effect on p21^{WAF1} stability[48]. 5-Azacytidine (5-Aza-C, a demethylating agent) mediated Sp1 expression also up-regulated activities p21^{WAF1}[53].

Rat-1 is a cell line containing wild-type p53[54]. Allan and co-w orkers found which p21^{WAF1} 5'UTR contains a putative CpG island which is m ethylated in Rat -1 cells that used frequently to assess transformation and for apoptosis studies, the lack of p21^{WAF1} expression appears to be the resul t of hypermethylation of the p21^{WAF1} promoter region, as p21^{WAF1} protein expression could be induced by growth of Rat-1 cells in the presence of 5 -aza-2-deoxycytidine(5-Aza-dC). Furthermore, sequencing analysis of bisulfi te-treated DNA demonstrated extensive methylation of cytosine residues in CpG d inucleotides in a CpG-rich island in the promoter region of the p21^{WAF1} gene[55]. A report showed that altered DNA methylation was present in RMS tumors and that the DNA methyltransferase expression is increased in both embryonal and alveolar subtypes of this cancer[56,57]. They think that hypermethylation of the p21^{WAF1} gene at the proximal STAT-binding site, xorrelates with decreased p21^{WAF1} expression. The p21^{WAF1} gene is su bjected to methylation regulation at the transcription level and is a target of aberrant methylation in RMS cells.

However, several studies indicated that the hypermethylation of p21^{WAF1} was not the main machineries of p21^{WAF1} expression regulation. Although Young *et al*[58] reported that cells arrested and p21^{WAF1} expressed by DNMT inhibition in normal human fibroblasts. Milutinovic demonstrated that i nhibition of DNMT resulted in the rapid induction of the known tumor suppressor and cell cycle regulator p21^{WAF1} by a mechanism that did not involve DNA m ethylation of the p21^{WAF1} promoter, in human non-small cell lung cancer c ell line, A549 cells[59]. The reduction of cellular DNMT protein levels also induced a corresponding rapid increase in the cell cycle regulator p21^{WAF1} protein demonstrating a regulatory link between DNMT and p21^{WAF1} which was independent of methylation of DNA[60]. Shin's result showed that the promoter of the p21^{WAF1} gene was not been methylated in gastric cancer cells. This confirmed that methylation was not the mechanism for inactivation of p21^{WAF1} in gastric cancer cells[20]. In adenomatoid polyps, alth ough DNMT1 expression coincided with the expression of other cell proliferation markers, many DNMT1-expressing cells also expressed p21^{WAF1}. The fidelity of DNMT1 expression was further undetermined in colorectal carcinomas, in which a striking heterogeneity in DNMT1 expression, with some carcinoma cells contain ing very high DNMT1 levels and others containing very low DNMT1. These results indicate that human colorectal carcinogenesis is accompanied by a progressive dys regulation of DNMT1 expression and suggest that abnormalities in DNMT1 expression may contribute to the abnormal CpG dinucleotide methylation which changes the characteristic of human colorectal carcinoma cell DNA[61].

HYPERACETYLATION, HDAC INHIBITORS AND OVEREXPRESSION OF p21^{WAF1} GENE

Histone deacetylation is a general mechanism for inactivation of the p21^{WAF1} in gastric cancer cell lines[20]. Both histone hyperacetylation and hypoacetylation appear to be important in the carcinoma process, and induction of the p21^{WAF1} gene by histone hyperacetylation may be a mechanism by which dietary fiber prevents carcinogenesis[31].

Regarding the correlation of histone acetylation and p21^{WAF1} gene

expression, that HDAC inhibitor TSA, trapoxin, butyrate and apicidin induce $p21^{WAF1}$ transcriptional activity involved in most studies.

TSA is originally reported to be a fungistatic antibiotic, and it appears to be a promising tool for analyzing the many functions of histone hyperacetylation in cell proliferation and differentiation. TSA can stimulate $p21^{WAF1}$ express ion in HT29 cells[32].

TPX is the microbially derived cyclotetrapeptide[62], Sambucetti found t hat it increased the level of chromatin acetylation associated with histone H3 in the trapoxin-responsive region of the $p21^{WAF1}$ promoter, and it activated $p21^{WAF1}$ transcription that led to elevated $p21^{WAF1}$ protein levels in three kinds of human tumor cells. Since the domain of the promoter that is ne cessary for TPX- mediated activation does not contain p53 binding sites, hence $p21^{WAF1}$ expression upregulation by TPX is independent of p53[30].

Sodium butyrate is a short chain fatty acid produced in the human colon by bacte rial fermentation of carbohydrates[32], causes hyperacetylation of histo ne through the inhibition of HDAC. Three years ago, Archer and his coworkers sho wed firstly the critical importance of $p21^{WAF1}$ in butyrate-mediated growth arrest was able to cause growth arrest in the human colon cancer cell line HT -29[31]. Siavoshian[32] suggested that butyrate and TSA stimula ted, the $p21^{WAF1}$ expression both at the mRNA and protein levels, whereas t hey induced histone H4 hyperacetylation. Butyate sensitivity requires Sp1-3 site in conjunction with the Sp1-5 site and Sp1-6[29]. Shin *et al* [20] indicated that the overexpression of $p21^{WAF1}$ gene occurred in human gastric cancer cell lines after butyrate treatment. Butyrate increased histo ne H4-acetylation in human melanoma cell lines A375 and S91 and up-regulated $p21^{WAF1}$ gene transcription level[63].

Apicidin is a fungal metabolite shown to exhibit antiparasitic activity by inhib ition of HDAC. Han *et al*[64] indicated that inhibition of HDAC activ ity by apicidin was closely associated with monorphological change and induction of $p21^{WAF1}$, although The protein levels of cyclin D1, CDK2, HDAC1 and p 53 were not affected by the addition of apicidin for 24 hrs, whereas the induc tion of $p21^{WAF1}$ by apicidin was reversible.

Suberoylanilide hydroxamic acid (SAHA) is a hydroxamic acid-based hybrid polar compound, and it is an inhibitor of HDAC[65,66]. SAHA causes an accumula tion of acrtylated histones H3 and H4 in total cellular chromatin by 2h, which is maintained throughout 24h of culture with increased $p21^{WAF1}$ expressi on, but no change in chromatin associated with the actin and p27 genes, and SAHA also induces up to a 9-fold increase in $p21^{WAF1}$ mRNA and protein in T24 bladder carcinoma cells. $p21^{WAF1}$ by SAHA is regulated, at least in par t, by the degree of acetylation of the gene-associated histones and that this i nduced increase in acetylation is gene selective[66]. These studies also suggest that $p21^{WAF1}$ is HDAC inhibitor and that the $p21^{WAF1}$ promoter is a useful model for study in hsitone acetylation regulated transcription.

In addition, MS-27-275 inhibits HDAC and causes hyperacetylation of histones, as well as induces the expression of $p21^{WAF1}$ various tumor cell lines[33].

The data above indicated that the induction of histone hyperacetylation by HDAC inhibitor is responsible for the antiproliferative activity through the crucial role of $p21^{WAF1}$ in the regulation of cell cycle.

PATHWAY OR FATORS ASSOCIATED TO ACETYLATION OF $p21^{WAF1}$

Several genes or transcriptional regulatory proteins including p300/CBP associate to $p21^{WAF1}$ gene regulation.

p53

The $p21^{WAF1}$ expression may be dependent[11,67] or independent of p53 regulation[68-70]. Also, the mechanisms of $p21^{WAF1}$ transcription regulation fall into two general categories: dependent or independent of the p53 gene[31]. The $p21^{WAF1}$ promoter contains five natural p53 binding sites, at positions 4001, 3764, 2311, 2276, and 1391, respectively (GenBank accession number U24170)[19].

p53 gene regulates the expression of $p21^{WAF1}$, and HDAC1,2,and 3 are al l capable of downregulating p53 function, i.e., interactions of p53 and HDAC2 likely result in p53 deacetylation, thereby reducing its transcription al activity[71]. Clark and co-workers found that loss of the G_1/S che ckpoint in HIV-1-infected cells may in part be due to Tat's ability to bind p53 and sequester its transactivation activity, as seen in both in vivo an d in vitro transcription assays[72].

$p21^{WAF1}$ overexpression has been seen to inhibit two critical checkpoints i n the cell cycle, G1 and G2, through both p53-dependent and -independent[74].

p300/CBP

Up to now, four families of nuclear proteins including p300/CBP and p300/CBP-as sociated cofactors contain an intrinsic HAT activity have been confirmed that po ssess HAT activity[74-78]. Accumulating evidences suggest that p300 and CBP are adaptors for various DNA-binding transcription factors[79]. Alt hough the precise mechanism by which p300/CBP stimulates transcription remains unclear, the discovery that p300/CBP and an associated factor P/CAF have histone acetylase activities suggests that these cofactors may regulate transcriptionth rough acetylation[80]. These activities have been proposed to modify the amino-terminal tails of the core histone ptoteins in a manner that may allow for some as yet uncharacterized modification of nucleosome structure.

p300 has been found to be required for induction of $p21^{WAF1}$ expression in keratinocyte differentiation[70]. Xiao and coworkers indicated the evide nces that p300 is required for TSA-induced, Sp1-mediated $p21^{WAF1}$ transcr iption: cotransfection of p300 elevated $p21^{WAF1}$ promoter activity, and thi s elevation was dependent on TSA-responsive GC-box; TSA-induced promoter acti vation was blocked by the introduction of p300 dominant-negative mutant into ce lls; Sp1- or Sp3- mediated activation was also suppressed by this p300 dominan t-negative mutant [28]. Owen *et al*[81] demonstrated the prog esterone regulated transcription of the $p21^{WAF1}$ gene through Sp1 and CBP/p300. A report[82] showed that $p21^{WAF1}$ stimulated trans-activatio n by p300/CBP, $p21^{WAF1}$ induction of p300 results from the activity of a di screte domain in the amino-terminal half of the protein which functioned to rep ress transcription. they proposed a model in which p300/CBP activity might switc hed between promoters following $p21^{WAF1}$ induced cell cycle arrest.

P/CAF and GCN5

Two human homologs of GCN5 have been cloned and shown to have HAT activity[83,84]. One homolog is human p300/CBP associated factor (hP/CAF), which is a transcriptional co-activator with intrinsic histone acetylase activity, which c ontributes to transcriptional activation by modifying chromatin and transcriptio nal factors[84,95]. The second family member is hGCN5[85,86].The ability of hGCN5 to acetylate nucleosomal histones is significantly re duced relative to its activity on free histones, where it predominantly modifies histone H3 at lysine 14.

The co-activator/adaptor protein GCN5 is a conserved histone acetyltransferase, which functions as the catalytic subunit in multiple yeast transcriptional regu latory complexes.

E2A

E2A gene encodes two alternatively spliced products, E12 and E47[87,88]. The $p21^{WAF1}$ promoter contains eight putative E-box consensus sequences, two of which lie between the TATA box and the transcription starting site, E2 and E1(as Figure 2). E1 binds E47 hetero- and homodimers and E2 has mush less aff inity for E47[89], and

it contains a conserved basic region responsible for DNA binding and a helix-loop-helix domain for dimerization[90].

TATA box E Boxes
TATATCAGGGCCG
CGCTGAGCTGCGC***CAGCTG***AGGTGTGAG***CAGCTG***CCGAAGTCAGT
E2 E1 |→+1

Figure 2 The nucleotide sequence of the p21^{WAF1} promoter from -149 through +1. There are the E1 and E2 binding sites.

E2A plays important roles not only in promoting cellular differentiation but als o in suppressing cells grown[89]. E2A binds to p21^{WAF1}, so the ove rexpression of p21^{WAF1} may be due to the effects of E2A transcriptional fa ctor[19]. Moreover, the overexpression of E2A proteins, such as E47 has been shown to induce p21^{WAF1} promoter activity independent of p53 bind ing sites[89].

Histone H4 but not histone H3 is acetylated from the endogenous p21^{WAF1} pr omoter in vivo, implying that CBP/p300, and not the SAGA complex is critical in complexing with E2A and upregulation of p21^{WAF1} in HTLV-1 infected cells[19].

The E3 box located 130 bp upstream from the TATA box also contributes to the act ivation of p21^{WAF1} expression, but the E4 to E8 boxes have no effect on p2 1^{WAF1} expression[89]. E2A is shown to be upregulated in HTLV-1 in fected T cells.

Sp1 binding

Sp family of proteins comprise ubiquitous and tissue-restricted transcription f actors that bind GC-rich DNA sequences and other related GT and GA motifs throu gh their zinc-finger domains[91].The ubiquitously expressed and closely related Sp1 and Sp3 factors have been found to regulate the promoters of severa l genes, including cell-cycle regulated genes, with Sp1 defined as a potent coo perative transcriptional activator and Sp3 as weak trans-activator or a repress or[91,92]. Sp1-binding sites appear to play a critical role in the main tenance of the methylation-free CpG island[93].Both Sp1 and Sp3 bind th e promoter of p21^{WAF1} gene[94].

The proximal p21^{WAF1} promoter contains a TATA box[81] and six Sp1 binding sites, also Sp1-1,-2,-3,-4,-5 and -6 near the TATA box[29]. p21^{WAF1} is Sp1 dependent promoters[95]. The region between -15 4 and transcription starting site contains Sp1-1,-2,-3,-4,-5,-6 binding si tes[20,81]. Sp1 is a sequence-specific transcription factor that recogn izes GGGGCGGGG and closely related sequences, often referred to as GC boxes. To Sp1, at last there are at least three homologous, transcription factors in the S p1 family: Sp2, Sp3 and Sp4[96]. Xiao also reported[28] TSA-ind uced promoter activation was blocked by the introduction of p300 dominant-negat ive mutant into cells. Their result from gel-shift assay[29] showed tha t physical and functional evidence which strongly indicated that both Sp1 and Sp 3 were responsible for TSA-induced transactivation for he murine p21^{WAF1} promoter in NIH3T3 cells. p21^{WAF1} gene is one of the natural target s of HDAC inhibitors. Sp1 is a sequence-specific transcription factor that reco gnizes GGGGCGGGG and closely related sequences, often being referred to as GC bo xes. To Sp1 at last there are at least three homologous, transcription factors i n the Sp family: Sp2, Sp3 and Sp4[96].

The GC-rich region in the six consecutive Sp1 binding sites of the p21^{WAF1} promoter was digested either with methylation-sensitive HpaII or with me thylation-insensitive MspI. The resulting DNA was subjected to a PCR react ion. Sp1 binding sites are the common elements that exist in the promoters of bo th genes[20]. Using transient reporter gene assays, Pagliuca et al[94] determined that Sp1 was a stroung activator of p21^{WAF1} promoter, whereas Sp3 functioned as a weak transactivator.

Signal transducers and activators of transcription (STAT)

STAT proteins recognize and bind to the palindromic sequence TTCNNGAA[95]. Such sequences have been identified in the p21^{WAF1} promoter region at nt -692,-2557 and -4232, and designated as sis-inducible element (SIE)-1, -2 and -3, respectively[97]. All three SIEs have been shown to bind STA T1. Chen *et al* indicated that hypermethylation of p21 gene at the proximal Sis-inducible element (SIE)-1, a STAT-responsive element located upstream of the p21^{WAF1} CpG 5-Aza-dC, demethylation at SIE-1 reactivated p21 ^{WAF1} expression[98]. STAT could up-regulate activation of cyclin-dep endent kinase inhibitor p21^{WAF1}[98,99].

In addition to its role in cell cycle regulation, p21^{WAF1} is also believed to inhibit DNA replication through its ability to bind proliferating cell nucle ar antigen (PCNA), which is required for both replicative DNA synthesis and DNA repair. However, p21^{WAF1} has no inhibitory effect on the DNA repair functi on of PCNA[100,101]. Thus, p21^{WAF1}may play a central role in preve nting the replication of mutations incurred after exposure of cells to DNA damage.

CONCLUSIONS

Histone acetylation is the major mechanism for regulation of the p21^{WAF1} gene in most cell lines (shown as Figure 3). Both histone hyperacetylation and hy poacetylation appear to be important in the carcinoma process. The influence of methylation on p21^{WAF1} gene expression is dependent on differentiation of cells and tissue. It is our anticipation that induction of the p21^{WAF1} gen e by histone hyperacetylation may become a mechanism of dietary prevention of carcinogenesis.

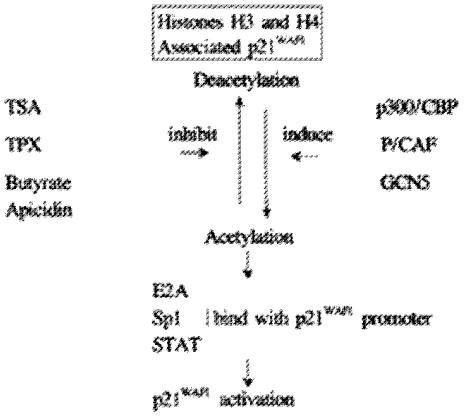

Figure 3 Butyrate,TSA,TPX and Apicidin inhibits HDAC enzyme ac tivity, and HAT from p300/CBP, P/CAF and GCN5 resulting in histone hyperacetylat ion of H3 or H4 associated p21^{WAF1} gene, and induces tran-scriptional activ ation of the p21^{WAF1} gene.

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