Small molecule modulators of epigenetic modifications: implications in therapeutics[+]

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The eukaryotic genome is organized into chromatin, a nucleoprotein complex and a dynamic entity that regulates the spatio-temporal expression of genes in response to the intracellular and extracellular signals. This dynamicity is maintained by several factors, including the chromatin modifying machineries. Chromatin modifying enzymes (for example, lysine (K) acetyltransferases for acetylation, lysine and arginine (R) methyltransferases for methylation, etc.) by virtue of their modifying abilities of both histones and the nonhistone components, are vital regulatory factors for gene expression both in physiological as well as pathophysiological conditions. Hence the modulators (inhibitors/activators) of these enzymes, which are capable of altering the gene expression globally, could also be useful in understanding the epigenetic mechanism of gene expression as well as for therapeutic purposes. We have found that acetylation of histone chaperone NPM1 and histones is essential for chromatin-mediated transcriptional activation. Remarkably, NPM1 as well as histones get hyperacetylated predominantly in oral cancer patient samples. We identified NPM1 as a positive regulator of the KAT, p300 autoacetylation, the possible causal mechanism of hyperacetylation. Targeting the acetylation by a water-soluble KAT inhibitor, CTK7A in oral tumour xenografted mice, we could demonstrate that the tumour growth could indeed be retarded upon the inhibition of KAT autoacetylation. Presently, we are studying the histone modification language in oral cancer, especially in the context of acetylation and methylation which could be potential targets for combinatorial epigenetic therapeutics.

Keywords: Chromatin dynamics, histone chaperone, lysine acetyltransferases, oral cancer, transcription regulation.

CHROMATIN is a complex three-dimensional organization of the eukaryotic genome composed of DNA, histones, nonhistone proteins and RNA components, which are intricately packed within the nucleus. The higher order organization of the genome is a deterrent to gene expression, since the elaborate machineries associated with the DNA templated processes require a decompacted open chromatin configuration to function. Hence, the chromatin organization is maintained as a dynamic feature which compacts and decompacts at specific regions based on the signals. The cell has evolved highly efficient complexes that aid in this constant opening and closing of the chromatin. These factors include the chromatin-associated proteins such as heterochromatin protein (HP1), positive cofactor (PC4), the histone chaperones (NPM1, nucleolin, Asf1, etc.), RNA such as the long noncoding HOTAIR, the ATP-dependent remodelling complexes such as Nuclear Receptor corepressor (NCoR), FACT and, most importantly, the chromatin modifying enzymes, an exhaustive list consisting of the reversible phosphorylation associated kinases-phosphatases, the acetylation associated acetyltransferases-deacetylases and the methylation associated methyltransferases-demethylases. These enzymes modify the histones as well as the nonhistone components of chromatin and thus are intricately involved in events associated with gene expression. The dysfunction of these enzymes has been identified in several disease conditions that lead to altered gene expression. Hence, the chromatin modifying enzymes have been identified as potential therapeutic targets against many diseases, including cancer. However, due to the general role of the chromatin modifying enzymes in modulating gene expression even in the normal cells, the specific targeting has been an important bottleneck in realizing the potential of 'epigenetic therapy'. The specific enzyme inhibitors and their targeted delivery have now made it possible to consider the epigenetic modulators along with the conventional therapeutics. Of the different research activities being carried out in our laboratory, a major

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component is the small molecule-based approach to understand biological phenomena as well as the exploitation of these modulators as potential therapeutic agents. In this article, we highlight one of our recent findings on oral cancer, where hyperacetylation of histones and histone chaperones has been identified as a causal factor. The mechanistic studies which led to the use of the acetyltransferase inhibitor as a tool to retard the oral tumour in a xenograft model will be highlighted. The progress of this work in the light of the present finding of other histone modifications in oral cancer and the potential significance of the arginine methyltransferase-specific inhibitor will also be briefly discussed.

Chromatin acetylation and cancer

Acetylation of chromatin is an important determinant of gene expression due to its chromatin decompaction ability, and also because of its role as a recruiter of chromatin remodelling complexes. The acetyltransferases are transcriptional coactivators and hence apart from their chromatin modifying activity also influence transcription by their co-activating function. The deacetylases are components of the repressive complexes and hence due to their multi-component nature also influence transcription at different levels. Due to the extensive involvement of the acetylation machinery in modulating transcription, they have deterministic roles in physiological as well as pathophysiological conditions, such as disease. Cancer is one such disease which exhibits these characteristic alterations. Since cancer is essentially an uncontrolled cell growth wherein the cells have lost their ability of cell cycle control, their overall functioning is still maintained by the same machineries of the cell.

The earliest example of altered acetyltransferase function in diseases has been related to cancer. These were due to the enzyme fusions wherein in the myeloid leukaemia cases, the MYST family of acetyltransferases was found to form fusions with altered acetyltransferase activities. Most of the acetyltransferases were identified to have mutations in several cancers and there were few reports of overexpression or increased activity of acetyltransferases in cancer[1]. A landmark work in the area of histone acetylation marks in cancers led to a profiling of various modifications in breast cancer and prostate cancer[2]. Later on with the advent of the high-throughput methods, the entire epigenetic modification profiling has been now attempted on several cancer cell lines as well as patient samples during different stages of differentiation. Recently, it was also reported that the histone H3K56 acetylation mark exhibits an increase in several cancers[3]. In this scenario, we initiated our research work on the most prevalent cancer in India, oral cancer, with respect to its altered epigenetic profile. Oral cancer in India has been attributed mainly to tobacco and betelnut chewing, partly to smoking, etc. However, the present status of oral cancer in India is as the topmost killer of Indian men compared to any other cancer-related mortality. Approximately, 12.8 males and 7.5 females per 100,000 were identified to be affected by oral cancer in a study reported in 2003 and the numbers have certainly increased since then[4].

As a preliminary characterization, we tested different cell lines for their hyperacetylation status and according to the earlier observations, we did observe an increased acetylation status in the hepatocarcinoma cell line. However, to our surprise we found that the histones in the oral cancer cell line KB were also hyperacetylated. The concept of histone hyperacetylation in cancer is a relatively new finding, since the earlier studies had showed a hypoacetylated state of histones with respect to cancer. However, the examined epigenetic mark was H4K16 acetylation, which is incidentally associated with DNA damage and repair process. Since the cancer cells are characterized by a loss in the cell cycle regulation ability, it is discernible that the epigenetic mark associated with this process could be lost.

We then decided to examine the oral cancer patient samples for their epigenetic alterations. The acetylation marks catalysed by p300 and PCAF: H3K9, H3K14 and the p300-specific acetylation mark, H2AK5 were also found to be hyperacetylated in the patient samples[5]. Incidentally, the acetylation on histone H4K16 was found to be decreased in accordance with the earlier reports[6]. To identify whether the hyperacetylation of the histones was due to an overexpression of the enzyme or due to the presence of the highly active enzyme, the samples were analysed for their p300 expression status as well as the auto acetylated p300 levels. It was observed that there was indeed a mild overexpression of p300 whereas the active enzyme form, autoacetylated p300 levels were found to be dramatically high. Thus, the hyperacetylation of histones could be partly due to the presence of the highly active acetyltransferase enzyme.

This led us to question the mechanism by which the autoacetylation of p300 was being induced. The auto acetylation of p300 is a process similar to the rate-limiting activation process in the case of the kinases. This has been mapped to a stretch of 62 residues (from lysine 1499 to lysine 1560) in the p300 acetyltransferase domain, and is represented by different residues, including the 12 lysine residues[7] which undergo transacetylation upon induction. This autoacetylation event precedes the acetylation of substrates. The autoacetylation can be induced by extracellular signals or by other proteins such as GAPDH. However, autoacetylation of p300 alters its structure[8], which is not only important for the hyperactivity of the enzyme but also efficient activation of transcription[9].

Another area of research work in the laboratory involves the elucidation of the role of chromatin-associated proteins and histone chaperones in transcription regulation.

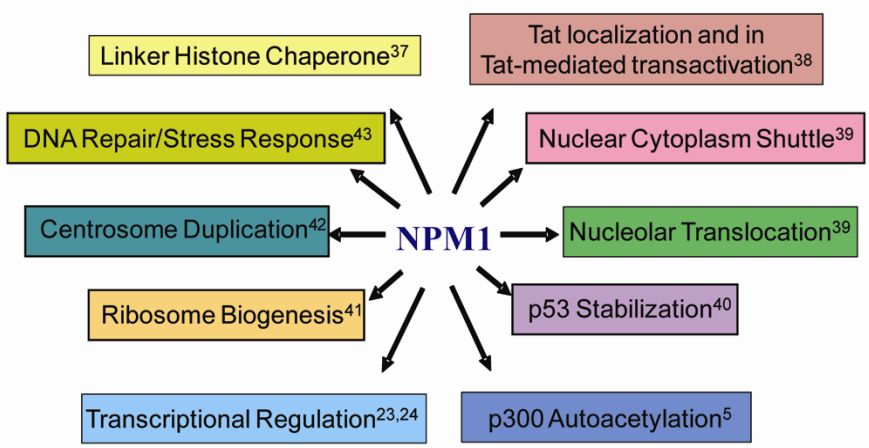

Figure 1. NPM1, a highly multifunctional protein shown to be involved in multiple cellular processes.

We found that the histone chaperone, NPM1 enhances transcription from the chromatin in an acetylation-dependent manner in which both NPM1 and histones get acetylated by p300. We were further interested in knowing the status and role of NPM1 in oral cancer.

Histone chaperones and cancer

Histone chaperones are histone-interacting proteins that are involved in all kinds of histone metabolism such as histone storage, transport, histone assembly as well as histone disassembly[10]. Due to their histone assembly and disassembly function, some of the histone chaperones such as the CAF-1 complex are essential during DNA replication in order to remove the histones ahead of the replication fork as well as to assemble histones into chromatin behind the replication fork. These histone chaperones are said to be replication-dependent due to the fact that they function only when DNA synthesis takes place. Histone chaperones also have a role in DNA repair and transcription due to the same requirement of chromatin assembly and disassembly during these processes. However, the histone chaperones involved in DNA repair are generally the replication-dependent chaperones as DNA synthesis is an essential step of repair. Whereas, histone chaperones involved in transcription are known to be replication independent[10].

Many histone chaperones have been implicated in different types of cancer mainly attributing to the fact that they are involved in replication coupled assembly. For example, the CAF-1 complex is required for replication coupled assembly and S phase progression and concurrently, it was found that the CAF-1 p60 levels (p60 is a subunit of the CAF-1 complex) were higher in proliferating cells compared to quiescent cells[11]. Further, CAF-1 p60 was found to be overexpressed in cells derived from breast tumours as compared to those derived from normal breast tissue. Based on these observations, CAF-1 p60 was suggested to be a strong candidate for a proliferation marker with an important prognostic value in breast cancers[11]. Further, another report showed that the CAF-1 p60 was overexpressed in prostate cancer and the degree of overexpression correlated with the aggressiveness of the cancer[12]. Similarly, Asf1b was expressed in cells in a proliferation-dependent manner, wherein the RNA and protein levels decreased when the cells exited the cell cycle. Depletion of Asf1b severely compromised proliferation and in accordance with these data, Asf1b mRNA levels were found to be higher in breast cancers and correlated well with disease outcome[13]. Another histone chaperone, tNASP is known to be a prognostic marker for several types of cancer, including breast, lung and prostate cancer[14]. The expression of tNASP is used as a serologic marker for ovarian cancer[15].

Multifunctional histone chaperones like NPM1 (Figure 1) and nucleolin are involved in regulating several diverse cellular processes and their alterations are correlated with cancer. The levels of NPM1 and nucleolin are correlated with the proliferative status of the cell. Nucleolin and NPM1 levels are higher in tumours and actively dividing cells[16–19] and are widely used as a marker of cell proliferation. The direct consequences of their overexpression on cell cycle are not clearly understood, but it is hypothesized that since both the proteins are involved in ribosome biogenesis, their overexpression may lead to stimulation of the same and thereby support the actively growing tumour cells. Interestingly, nucleolin was found to be present on the cell surface of cancer cells, which led to the identification of nucleolin as a diagnostic marker for cancer cells[20]. NPM1 plays a role in cancer development through multiple mechanisms. The chromosomal translocations that lead to NPM1 N-terminal fusion to certain transcription factors like ALK, RARα, MLF1, etc. are common in some types of lymphomas or leukaemias[21]. Its C-terminal mutations have been reported in

acute myelogenic leukaemia (AML), wherein the nucleolar localization signal is disrupted and a new nuclear export signal is generated due to which the protein localizes mainly to the cytoplasm. The deletion of 5q, the genetic loci which encodes NPM1 leads to myelodysplastic syndrome (MDS)[21]. The loss of NPM1 in this case might cause genomic instability due to unrestricted centrosome duplication[22]. All these findings indicate towards a role of NPM1 as a tumour suppressor. However, overexpression of NPM1 has been noted in various tumours, and it has been proposed as a marker for gastric, colon, ovarian and prostate carcinomas. These findings implicate NPM1 as a proto-oncogene[21].

NPM1 and oral cancer

NPM1, being implicated and known to be overexpressed in cancer, led us to study the levels of NPM1 in oral cancer. We found by Western blotting, immunohistochemistry (IHC) and RT–PCR analysis, that indeed NPM1 was overexpressed in oral squamous cell carcinoma (OSCC). NPM1 was found to be dynamically acetylated by p300 acetyltransferase, but not by its closest homologue, CBP and deacetylated by SIRT1. Since p300 was also found to be slightly overexpressed and highly autoacetylated in oral cancer patient samples, we went on to study the levels of acetylated NPM1 in oral cancer. We found enhanced acetylation levels of NPM1 in oral cancer patient samples by Western and IHC analysis and the levels of acetylation increased in a grade-dependent manner. However, when we similarly studied the levels of SIRT1 in the same patient sample, we did not find any alteration in the expression of the deacetylase[23]. Presumably, p300 overexpression combined with its hyper autoacetylation induced by different signals might be responsible for the enhanced acetylation status of NPM1 (Figure 2).

Interestingly, the acetylated NPM1 was found to be present in the nucleoplasm instead of the nucleolus where majority of the NPM1 is known to reside. Using co-immunofluorescence analysis in an oral cancer cell line, KB cells, we found that acetylated NPM1 perfectly colocalizes with active RNA polymerase II transcription foci. In an earlier work, we showed that NPM1 enhances *in vitro* transcription from the chromatin template in an acetylation-dependent manner[24]. Possibly, acetylated NPM1 associates with active RNA polymerase II and promotes transcription by an unknown mechanism. To partially test this hypothesis, we performed siRNA-mediated knock down of NPM1 in KB cells, followed by a microarray analysis. It was revealed that knock

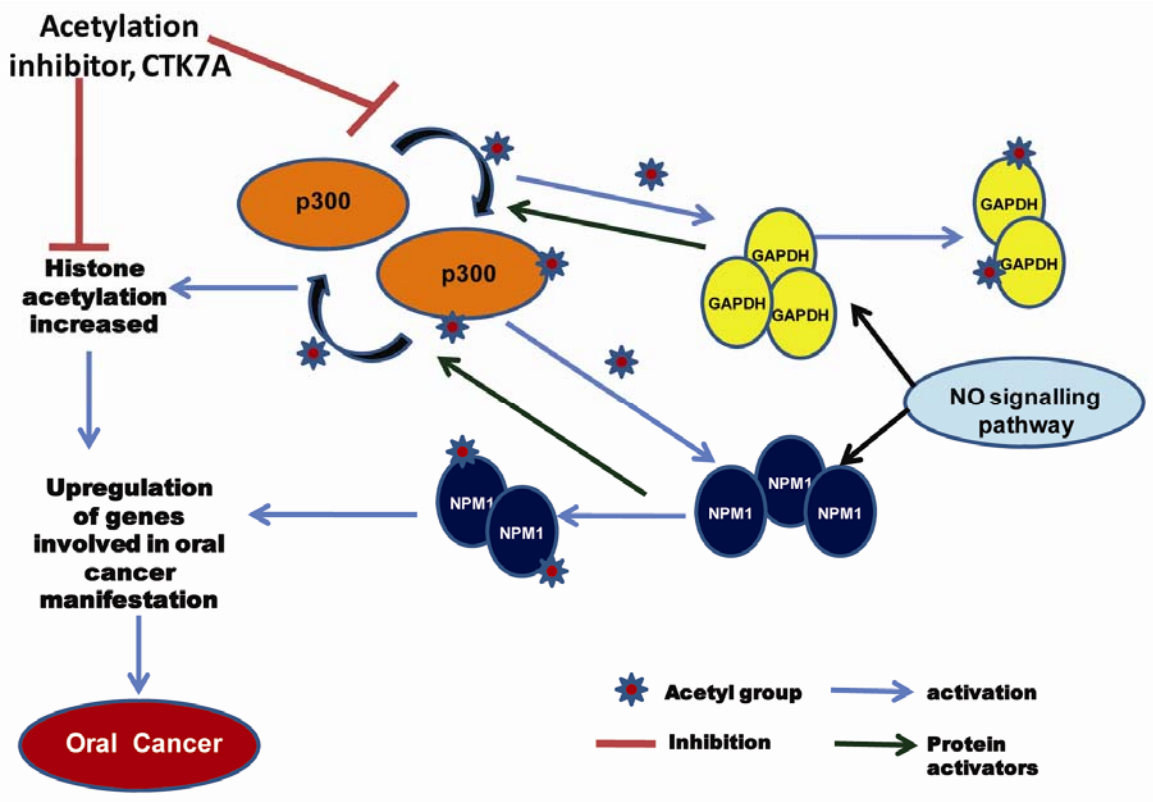

Figure 2. Nitric oxide signalling induces the overexpression of GAPDH and NPM1, which induces the autoacetylation of p300. The highly active autoacetylated p300 hyperacetylates the histones and NPM1, which leads to upregulation of genes involved in oral cancer manifestation. CTK7A inhibits the autoacetylation of p300 and thereby its HAT activity leading to inhibition of gene expression and subsequently inhibiting the proliferation and growth of oral tumours.

down of NPM1 led to differential regulation of several genes involved in diverse cellular processes. The major genes that were downregulated belonged to that of ribosome biogenesis, proliferation and oxidative phosphorylation pathways, whereas genes involved in focal adhesion, adherens junctions, insulin signalling, and histidine and butanoate metabolism were significantly upregulated. Additionally, some of the genes involved in apoptosis and cell death were upregulated. Apart from the global effect on gene expression, several genes implicated in cancer manifestation were also found to be downregulated upon knock down of NPM1. Significantly, we found that the expression of genes like those for TNF-α, the TNF receptor, the interleukin-6 (IL-6) receptor, and IL-6ST (the IL-6 signal transducer) were reduced and that of CTNNB1 (beta-1 catenin) was increased as a consequence of NPM1 silencing. CTNNB1 encodes one of the components of the cell–cell adhesion complex, suggesting a possible role for NPM1 in facilitating the metastasis process during carcinogenesis. Interestingly, it has been reported earlier that TNF-α, IL-6 and IL-6 receptor levels increase substantially in OSCC. These findings proved that NPM1 influences the expression of genes involved in cancer. To further test whether these genes were directly regulated by NPM1, ChIP-on-chip analysis was performed to study the occupancy of NPM1 across all the known human gene promoters. We found that NPM1 was enriched in a number of human gene promoters, such as those for IL-encoding genes, the TNF-α encoding gene. Higher levels of acetylated NPM1 in cancer would possibly result in its increased occupancy at such gene promoters. We selected genes which were downregulated upon NPM1 knock down and were directly regulated by NPM1 as revealed by its occupancy in the ChIP-on-chip analysis, one such candidate gene was TNF-α. ChIP assays revealed that acetylated NPM1 occupies the TNF-α promoter in KB cells. A ChIP assay done after siRNA-mediated knock down of NPM1 resulted in reduced recruitment of RNAP II on the TNF-α promoter compared to that in control siRNA-transfected cells. reChIP assays in which the first chromatin pulldown was done with either an anti-NPM1 or an anti-AcNPM1 antibody, followed by an anti-RNAP II antibody, showed that acetylated NPM1 and RNAP II colocalized at the TNF-α promoter. Similar set of experiments was performed with the anti-phosphoserine5 RNA Pol II antibody which marks actively transcribing RNA Pol II. These experiments showed that acetylated NPM1 associates with active RNAP II at the promoter of TNF-α, which results in its enhanced expression. Thus, the acetylation status of NPM1 could be involved in oral cancer manifestation through the activation of critical genes required for cell proliferation and survival. The positive correlation of the grade of cancer with the acetylation levels of NPM1 may lead to the development of acetylated NPM1 as a new marker for prognosis as well as a possible therapeutic target[23].

Mechanism of p300 autoacetylation in oral cancer cells

Several signalling pathways have been identified to be responsible for the manifestation of different cancers. One such signalling molecule is the pleiotropic, nitric oxide (NO), which is known to induce the interferon-γ (INF-γ) pathway via activation of the inducible nitric oxide synthase (iNOS). Since signalling molecules lead to activation of several downstream pathways, we tested whether NO production leads to an overexpression of the histone chaperone, NPM1 and the metabolic enzyme, GAPDH. Indeed in the cell-culture conditions when a NO donor was used, an overexpression of both NPM1 and GAPDH was observed. Incidentally, NO signalling has already been shown to induce GAPDH expression. Another independent study had earlier reported that NO-induced GAPDH can enhance p300 autoacetylation[25]. Similarly, the NO-dependent NPM1 increase also led to an enhancement of p300 autoacetylation. This was also found to be true in the case of the oral cancer patient samples which exhibited increased levels of iNOS by immunohistochemical analysis. Thus, in oral cancer, the NO-mediated IFN-γ signalling pathway led to an induction of expression of NPM1 and GAPDH, which acted as protein enhancers of p300 autoacetylation. This event of autoacetylation led to a highly active p300 leading to hyperacetylation of histones, histone chaperones and other proteins. Acetylated NPM1 regulates transcription of TNFα, thereby leading to activation of the downstream targets, thereby resulting in cancer manifestation (Figure 2).

Histone acetyltransferase inhibitor, CTK7A

The activation of the acetyltransferase activity has been identified as an important causative means for oral cancer. Hence, the use of acetyltransferase inhibitors should be able to modulate the cancer progression process. A major research area spanning a decade in the laboratory has been the identification of specific modulators of acetyltransferases and other chromatin modifying enzymes. Earlier we had identified anacardic acid from cashewnut shell liquid as a potent inhibitor of acetylation[26]. However, its reduced bioavailabilty as well as its increased potency led to the search for better inhibitors. We identified garcinol from *Garcinia indica* (Kokum) as a potent acetylation inhibitor[27]. This was found to be toxic to mammalian cells. Subsequent derivatization of this compound led to the synthesis of LTK14, a p300 acetyltransferase-specific inhibitor[28]. Another study in our laboratory led to the identification of curcumin as a p300 acetyltransferase-specific inhibitor[29]. The bioavailability of curcumin has been a long-standing problem, which has been tried to be overcome by different modifications, including nanomechanism-mediated delivery[30]. We decided

to synthesize a water-soluble derivative of curcumin and we obtained the sodium salt of the same which had inhibitory potential against acetyltransferases p300 and PCAF.

The water-soluble inhibitor, CTK7A when tested in the oral cancer cell line, KB, led to hypoacetylation of histones. The inhibitor treatment also led to a reduction in the proliferative potential of the cells and induced senescence-like growth arrest as exhibited by the β-gal staining. In the cells, additionally, the effect of CTK7A on wound healing was also tested. Upon inhibitor treatment there was a compromise in the wound-healing ability. This is an important characteristic in the process of cancer progression, since wound healing essentially translates into angiogenesis in cancerous tissues. Therefore, any inhibitor capable of compromising the wound-healing ability of cells can have potential anti-angiogenic effects. Since CTK7A showed an anti-proliferative effect as well as senescence-like growth arrest, and also a decrease in wound-healing ability, the possibility of CTK7A to have anti-cancer activity on the oral cancer cells was tested on the nude mice model xenografted with KB cells. Convincingly, the oral tumour exhibited growth retardation in the animals treated with CTK7A. The tumour tissue treated with CTK7A also showed a decreased level of acetylation of histone H3K9, K14 albeit without any alteration in the p300 levels. This is also co-related with a decrease in the levels of NPM1, GAPDH, iNOS and COX2 in the treated tumour tissues[5] (Figure 2). Thus, it is indeed clear that the acetylation modification plays an important role in the process of oral cancer manifestation, which can be addressed using an acetyltransferase inhibitor. Since CTK7A is a water-soluble compound, its apparent toxic effects are minimal. Furthermore, it has a broad-spectrum acetylation inhibition potential. Thus, CTK7A could be a potent lead molecule for anti-neoplastic therapeutics. However, a detailed characterization of the bioavailability of CTK7A and its metabolic fate are presently being studied in the laboratory.

Epigenetic language in oral cancer

A recent concept in the field of epigenetics is the 'epigenetic language'. Several interesting studies have laid down the foundation for the understanding of how the epigenetic modifications influence gene expression. One of the common themes of most of these studies is the co-existence of several modifications together for a particular expression pattern. Thus, epigenetic language can be defined as the combination of different chromatin modifications that are regulated in a spatio-temporal manner in response to specific signals. However, not all modifications are present on all promoters at all times; rather there is a fine-tuning of the appearance of these modifications[31]. For example, a normal transcriptional activation process is characteristically represented by histone acetylation on H3K9, K14 as well as H3K4 trimethylation[32]. But, in the case of a transcriptional activation process in response to DNA damage, apart from the aforementioned modifications, the H4K16 acetylation and H3K56 acetylation marks are also observed on promoters. Arginine methylation on H3 and H4 tails also exhibits such differential specificities. The crosstalk of histone modifications in different physiological processes has been reviewed recently[33].

The MLL methyltransferase-mediated H3K4 trimethylation is closely linked to the p300-mediated H3 and H4 acetylation. With respect to the arginine methylation, an intricate network exists wherein PRMT1-mediated histone H4 R3 methylation acts as an activation mark for p300-mediated H3 and H4 acetylation. Such acetylated histone tail is a preferred substrate for CARM1-mediated H3R17 and R26 methylation. Histone tails modified in this manner have been shown to facilitate p53-mediated transcriptional activation[34]. There are many examples of such crosstalk of epigenetic modifications which cooperate in a coordinated manner to facilitate gene expression. Since in oral cancer, the p300 activity is altered and thereby the histone acetylation marks also show an increased expression, it is hypothesized that other transcription activation marks such as H3K4 trimethylation, H3R17 and R26 methylation may also show an altered expression in oral cancer. In this scenario, it is also necessary to develop modulators for histone methylation.

Histone methylation modulators

As mentioned earlier, work in the laboratory also involves the identification of modulators of other chromatin modifying enzymes such as the methyltransferases. During our routine screening procedure, we identified the crude extract of pomegranate fruit rind to possess potent inhibitory activity against arginine methyltransferase, CARM1/PRMT4 and acetyltransferase p300. Subsequent purification of the components of the pomegranate crude extract led to the isolation of ellagic acid (TBBD), which when tested in the *in vitro* assays exhibited specificity towards coactivator associated arginine methyltransferase, CARM1. Further characterization of TBBD-mediated CARM1 inhibition suggested a unique substrate sequence-dependent enzyme inhibition mechanism. CARM1 can methylate histone H3 at R17 and R26 residues with physiological consequences. The histone H3 tail has a proline preceding the R17 residue, which by virtue of its structural characteristic allows a docking site for TBBD in the enzyme-substrate (ES) complex, thereby preventing the methylation of the R17 residue. On the other hand, the R26 residue which is preceded by an alanine at the 25th position, is not affected by TBBD. The significance of the proline residue in determining the inhibition was verified using site-directed mutagenesis

strategy, wherein the proline residue was replicated at the 16th as well as 25th position, which showed an inhibition pattern for both R17 and R26 methylation. However, another mutant which had alanine residues at the 16th and 25th position did not exhibit any inhibition in the presence of TBBD. This was also verified by employing the isothermal calorimetric titrations, wherein the binding sites on histone H3 in the proline-containing mutant were more compared to the wild type. Similarly, the alanine-containing mutant had lost its binding sites with respect to the wild-type protein. Thus, TBBD was identified as a unique H3R17 methylation-specific inhibitor[35] and hence is presently being used as a biological probe to understand the role of H3R17 methylation in physiological conditions.

Combinatorial therapy

Since the process of transcription and gene expression is regulated by a set of epigenetic marks, and these altered gene expression patterns are the target for epigenetic therapy, it is evident that targeting a single modification may not lead to a complete therapeutic effect. Rather, the targeting of different modifications by the specific modulators would lead to a better therapeutic effect.

Nanomaterial-mediated delivery

Recently, work in the laboratory has also extended to the area of nanobiotechnology, wherein we have utilized glucose-derived carbon spheres for delivery of a cell-impermeable small molecule with the ability to activate histone acetylation. The first identified, natural, small molecule inhibitor of acetylation, anacardic acid[26] was highly toxic and hence was derivatized in order to achieve an efficient and non-toxic molecule. However, the derivative, CTPB obtained turned out to be an activator of acetyltransferase rather than an inhibitor. The identification of this molecule was an important step towards direct activation of acetyltransferase[26], since the only other way of activating acetylation by the small molecules was using the deacetylase inhibitors, which are more non-specific in nature. But, the fact that CTPB was not permeable to cells dampened the significance of this finding. Subsequently, we identified the carbon spheres (CSP) which were intrinsically fluorescent and had the ability to cross the cell membrane as well as the nuclear membrane to be localized in the nucleus in a time dependent manner. Therefore, we decided to test the utility of CSP as a delivery vehicle for CTPB. Since the CSP is characterized by several functional groups, we adopted a normal adsorption method, and the presence of CTPB on CSP was confirmed by performing field emission scanning electron microscopy (FESEM) and elemental energy dispersive X-ray spectroscopy (EDAX) analysis. These CTPB–CSP conjugates when tested on the mammalian cell line could now exhibit hyperacetylation, indicating that the activator was working in the cellular mileu. Subsequently, the CTPB–CSP conjugate could also induce hyperacetylation in the mice brain[36]. Surprisingly, the CSP was also capable of crossing the blood–brain barrier possibly due to its origin from glucose. The most significant observation from this study was the tenfold decrease in the compound concentration upon conjugation with CSP. The acetyltransferase inhibitor, CTK7A described earlier, is used at concentrations above 100 μM for achieving any significant effects. Hence the potential use of CSP as a targeted delivery agent with reduced compound concentrations needs to be studied with the inhibitors as well.

Future perspectives

The histone modification language for different sets of genes and signals is presumably not the same. It would be essential to elucidate this language for our better understanding of physiological or pathophysiological phenomenon regulated by different epigenetic signals. In this context, chemical biology approach using small-molecule modulator of epigenetic enzymes could be the best choice. These chemical tools would be extremely useful to design therapeutically important lead molecules for diseases such as cancer, AIDS and diabetes.

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