Histones: Controlling Tumor Signaling Circuitry

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Abstract

Epigenetic modifications constitute the next frontier in tumor biology research. Post-translation modification of histones dynamically influences gene expression independent of alterations to the DNA sequence. These mechanisms are often mediated by histone linkers or by proteins associated with the recruitment of DNA-binding proteins, HDAC I and II interacting proteins and transcriptional activators, coactivators or corepressors. Early evidence suggested that histones and their modifiers are involved in sophisticated processes that modulate tumor behavior and cellular phenotype. In this review, we discuss how recent discoveries about chromatin modifications, particularly histone acetylation, are shaping our knowledge of cell biology and oncogenic circuitry. We explore the role of histones and their modifications in the control of normal tissue homeostasis through maintenance of tissue-specific genetic signatures. However, deregulated environmental cues can influence cellular transformation by altering local signaling circuitry, resulting in dysfunctional epigenetic equilibrium. By interacting with environmental cues, tumors undergo genetic deviations resulting in changes on cancer behavior, cellular phenotype, increase aggressiveness or induction of tumor dormancy. Therefore, it is important to understand the crosstalk between environmental signals and tumor behavior in epigenetics.

Keywords: H3; H4; Oral cancer; Whole genome sequencing; Genetic code; Histone modifications; Acetylation; Chromatin compaction

Introduction

With the completion of the human genome it becomes evident the upcoming opportunities to explore and identify new genome-scale mechanisms involved in gene expression. The field of epigenetics presents a unique opportunity to understand the molecular mechanisms underlying the modulation of gene expression in the absence of alterations to the DNA sequence. Epigenetics is involved in the control of normal tissue homeostasis through maintenance of tissue-specific genetic signatures. However, deregulated environmental cues can influence cellular transformation by altering local signaling circuitry, resulting in dysfunctional epigenetic equilibrium. By interacting with environmental cues, tumors undergo genetic deviations resulting in changes on cancer behavior, cellular phenotype, increase aggressiveness or induction of tumor dormancy. Therefore, it is important to understand the crosstalk between environmental signals and tumor behavior in epigenetics.

Oncogenomics, which uses high-throughput technologies to screen cancer-associated genes, is a novel strategy for tackling the complex interaction between DNA mutations and epigenetic driven deviations. DNA mutations originate from changes in the nucleotide sequence mainly derived from insertions, deletions, point mutations and chromosomal translocations that influence the normal balance between cellular proliferation and death. In an effort to identify new biomarkers and develop novel cancer therapeutics, multiple mega oncovalence projects were initiated. These included the Cancer Genome Project at the Wellcome Trust Sanger Institute and the Cancer Genome Anatomy Project (CGAP), a National Cancer Institute initiative aimed at determining the genetic expression of normal, precancerous, and cancerous cells.

In the last several years, oncovalence applications have extended from identifying genes associated with cancer to analyzing transcription abundance in tumors (Transcriptome) [1,2]. DNA methylation (Methylome) and histone acetylation (Acetylome) [3,4]. The transcriptome is highly influenced by histone modifications that are primarily driven by acetylation and methylation. Histone modifications directly influence chromatin DNA packaging, resulting in a decondensed structure and enhanced interactions with transcription factors. This process, referred as the histone code, confers a high degree of cellular plasticity in both normal cells, such as the neuron synapse and lymphocytes [5,6], and tumor cells of the ovary and head and neck [7-9]. We highlight below how histones and its modifications are involved in stem cell biology and in the molecular circuitry governing tumor progression. We also present and discuss the involvement of histone acetyltransferase, histone deacetylases and its inhibitors as a therapeutic strategy used in various cancers. Further, we explore recent findings on head and neck cancer mutations of histones and histone modifiers identified using next-generation sequencing.

The Chromatin: Epigenetic Switches of the Genome

The genome is organized and packed into the nucleus through interactions with core histone proteins. Differential DNA folding regulates access to regulatory elements, resulting in alterations of the cellular response to environmental cues. DNA folding modifies cellular phenotypes by inducing cell type-specific chromatin organization [10]. We recently reported on functional chromatin folding in head and neck squamous cell carcinoma (HNSCC) cell lines during tumor response to environmental cues that dynamically modulates tumor behavior and cellular phenotype [9].

The ability of the chromatin to modulate cellular behavior is associated with how tightly DNA is spooled around H2A, H2B, H3 and H4 core histones [11]. Together, histones and DNA form nucleosomes, the fundamental units of chromatin. Each nucleosome is composed

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of 146 bp of DNA coiled in sequence around a core of eight histone proteins [12].

Histones, the most abundant proteins associated with DNA, were highly conserved during evolution and are associated with the regulation of nuclear gene expression in several tissue types. Histones maintain contact with DNA through their flexible globular domain and amino acid residue regions (lysine, serine and arginine) that protrude from the nucleosome (histone "tail" domain). The majority of post-translational modifications in histones, such as lysine acetylation, serine phosphorylation, lysine methylation, arginine methylation, and lysine ubiquitination, occur at the residue region [13-17]. The pattern of histone modifications determines chromatin status (euchromatin or heterochromatin) [18], the accessibility of DNA to nuclear factors and ultimately transcription. Euchromatin is relatively uncondensed and represents loci being actively transcribed. In contrast, heterochromatin is condensed and represents areas of the genome that are silenced. Several studies show that alterations in chromatin structure due to histone modifications correlate with gene expression, the cell cycle, DNA replication and damage, DNA repair and chromosome stability [19,20].

Dynamic changes in chromatin structure organization are mainly driven by histone acetylation and deacetylation, mechanisms responsible for important regulatory functions of gene expression. These processes are controlled by histone acetyltransferases (HAT) and histone deacetylases (HDACs), enzymes that are targeted to specific amino acid positions. In histone acetylation, HATs catalyze the addition of an acetyl group to the lysine residue in the tails of histones resulting in neutralization of the positive charge in the histone tail. The new acetyl group reduces the histone-DNA interaction, resulting in open or active chromatin that allows binding of transcription factors to promoters of target genes. Therefore, increased levels of histone acetylation are generally associated with increased transcriptional activity [21]. Acetyl groups are removed in histone deacetylation, resulting in increased histone-DNA interaction and chromatin condensation that blocks transcription by preventing the binding of transcription factors and regulatory complexes [21-23].

HATs and HDACs act in a coordinated manner to influence gene expression in different tissues. These enzymes induce conformational changes in histone-DNA interactions that alter the accessibility of transcription factors to DNA and are crucial epigenetic changes for the development and differentiation of many normal cell types. However, altered HAT and HDAC activity has also been observed in several types of cancer and may be promising therapeutic targets for these diseases [13,21,24].

Epigenetic regulation of gene expression is primarily controlled by histone acetylation and methylation, histone modifications, and changes in nucleosome conformation [20]. There is growing evidence that epigenetics is involved in gene regulation during mammalian development (reviewed in [25]). These processes are observed during the maintenance of embryonic stem cells (ES) in the early stages of embryogenesis. Polycomb group (PcG)-protein repressive system is an example of epigenetic control of gene expression. PcG form a multiprotein complex involved in chromatin organization and capable of epigenetically induce modifications on its structure thereby influencing gene transcription. In ES cells, PcG repressive system suppresses developmental pathways, thereby maintaining ES cell pluripotency and plasticity during embryonic development [26]. Furthermore, Boyer et al. identified several PcG target genes in ES cells bound by OCT4, SOX2 and NANOG, three transcription factors associated with pluripotency [27]. Similarly, the inability of the PcG-protein repressive complex to suppress developmental genes in ES cells results in spontaneous cellular differentiation [26]. In accordance with the PcG-protein repressive complex, the effect of HDAC on chromatin organization regulates the maintenance of stem cell pluripotency in coordination with numerous signaling pathways [28,29]. In contrast, chromatin condensation in tumors is associated with chromesistance [30-33] through the reactivation of stem cell-like transcription programs [34]. In addition to the known functions of the PcG-protein repressive complex, recent reports have shown that the histone variant H2A.Z plays a similar role in early development. H2A.Z assists with the dissociation of H2A-H2B dimers, resulting in modifications to the nucleosome surface that promotes transcription factor binding [35]. H2A.Z induces changes in chromatin configuration allowing access to transcription factors and cofactors that activate and repress regions in embryonic stem cells leading to self-renewal and differentiation [36,37]. This mechanism involves the depletion of nucleosomes at the Transcription Start Sites (TSSs) where gene transcription begins. Recently, Schones et al. found that nucleosomes containing H2A.Z are particularly susceptible to depletion [38]. Further, Li et al. observed by high-resolution nucleosome mapping and chromatin immunoprecipitation sequencing (ChIP-Seq) that co-occupancy of the transcriptional activator FoxA2 and H2A.Z results in nucleosome depletion during ES cell differentiation, coinciding with gene activation [37].

**Histone Acetyltransferase (HAT) and Histone Deacetylases (HDAC)**

The equilibrium between histone acetylation and deacetylation in the promoter region of chromatin is essential for the regulation of gene expression and the establishment of a transcriptionally competent chromatin state [39-42]. The antagonistic effects of HDACs and HATs tightly control the balance of steady-state acetylation. While HDACs promote transcriptional repression by removing acetyl groups from lysine, HATs increase transcriptional activity through the addition of acetyl groups. Besides these electrostatic changes, HATs and HDACs also play roles in gene activation or silencing through the recruitment of co-activators and co-repressors of transcription.

Alterations in histone locus-specific post-translational modifications are proposed for describing a code, which defines the state transcriptional potential of a cell referred as the code of histones. A global comprehension of this code is necessary for dissecting the role of histones in physiology and pathology [43]. Initial steps towards this goal have been made with the characterization of the histone deacetylase-1 enzyme (HDAC1) as a key regulator of embryonic stem cells proliferation and repression of cell cycle inhibitors in mice indicating its requirement during embryonic development [44]. Impairment of HAT activity or overexpression of HDAC by aberrant gene expression is associated with the process of carcinogenesis and tumor progression [13,21,45,46]. Because histone modifications do not alter the DNA sequence, new agents have been developed that reverse this modification, resulting in reactivation of tumor suppressor genes and reestablishment of a signaling balance to maintain cellular homeostasis. Notably, inhibitors of histone deacetylases (HDACi) are a new group of anticancer agents that have shown promising results [47].

**Histone acetyltransferase (HAT) and its Inhibitors**

The HATs are divided into the following five distinct families...
Histone deacetylases (HDACs) and HDAC inhibitors

HDACs are cellular enzymes expressed in many tissues that play important roles in differentiation, proliferation, and cancer. HDACs are primarily involved in the acetylation network, and several HDACs have been described in humans. Based on similarities, HDACs are divided into four functional classes based on sequence homology to their corresponding transcriptional regulator of yeast counterparts (Saccharomyces cerevisiae): 1) class I (HDAC1, HDAC2, HDAC3 and HDAC8); 2) class II is subdivided into class IIa (HDAC4, HDAC5, HDAC7, and HDAC9) and class IIb (HDAC6 and HDAC10); 3) class III (SIRT1 to SIRT7); and 4) class IV (HDAC11 and related enzymes) [13,39,69,70]. These enzymes are part of multi-protein complexes, and their activity depends on other factors, such as mSin3, N-CoR and SMART transcriptional co-repressors. Changes in these factors can indirectly interfere with HDAC activities [21].

HDACs also have numerous non-histone target proteins comprised of transcriptional regulators (Rb, DEK, HMG1(Y)/HMGAI and others), transcription factors (p53, c-Myc, BCL-6, E2F1 and others), DNA repair enzymes (Ku70, TDG, NEIL2 and others), nuclear import regulators (Rch1 and importin-α7), chaperone proteins (HSP90), structural proteins (α-tubulin), viral proteins (E1A and others) and inflammatory mediators (HMGB1) [71]. These proteins are involved in cellular proliferation, differentiation and cell death, and their functions can be intrinsically modulated by protein acetylation [23,71].

Class I HDACs are ubiquitously expressed in all tissue types [72,73]. Of particular interest for this review, HDAC1 and HDAC2 are involved in proliferation, regulation of the cell cycle and apoptosis. Posttranslational modifications of HDAC1 and HDAC2 alter their enzymatic activity, subcellular localization, stability, and interaction with other binding sites in response to different stimuli. In addition, deregulation of HDAC1 and HDAC2 is associated with the onset of many diseases in humans, including cancer [73]. HDAC1 suppresses activity of the p21WAF/SIP1 (cyclin-dependent kinase inhibitor) pathway by deacetylating lysine 4 residues on histone H3 [73,74]. Acetylation of HDAC1 dramatically reduces its enzymatic and repressive functions in vitro and in vivo, and overexpression of HDAC1 has been observed in many epithelial malignant tumors, such as hepatocellular [75], colorectal, gastric [76], pancreatic [77], and prostate [32] carcinomas. In contrast, depletion of HDAC1 results in perturbation of the cell cycle, loss of mitotic cells and increased cell death in several human cancer cell lines [78]. HDAC2 is overexpressed during the polyp stage of familial adenomatous polyposis colon cancer [79]. High levels of HDAC2 are also found in cervical dysplasia and invasive carcinomas [77]. Increased expression of HDAC1, HDAC2 and HDAC3 is associated with advanced stage prostate, gastric and colorectal cancers [76].

SIRT1, a member of the class III HDACs, acts as an important regulatory route capable of influence the apoptotic machinery. SIRT1 represses DNA damage-induced apoptosis through deacetylation of p53 [80]. SIRT1 also interferes with activation of apoptosis by deacetylating FOXO3a and/or E2F1 to inhibit their transcriptional activity [81,82]. Along with the control over the apoptotic machinery, SIRT1 promotes tumorigenesis by inhibiting cellular senescence, impairing cellular differentiation and promoting cellular growth associated with angiogenesis. SIRT1 is found highly expressed in many cancers, including acute myeloid leukemia, prostate carcinoma, and colon carcinoma, among others [83]. Further, SIRT1 is known to induce tumor resistance to chemotherapy suggesting its role as an oncogene (review in [87]). In contrast, SIRT1 is also considered a tumor suppressor gene due to its role in genomic stability, in limiting the replicative life span, in regulate cellular response to genotoxic stress and in the suppression of intestinal tumorigenesis [84-86]. Together, contradictory reports on SIRT1 function strongly suggests distinct roles depending on the involved signaling machinery.

HDACs inhibitors (HDACi) constitute a novel and growing family of potent anticancer agents that activate or repress 4-12% of genes by promoting histone acetylation, relaxing DNA that is coiled around core histones and increasing access of DNA to transcription factors [48,88-92]. In cancer cells, HDACi impair survival pathways resulting in cellular differentiation mediated by cell cycle arrest, block angiogenesis, induce apoptosis by stimulating the death receptor and...
intrinsic apoptotic pathway, and prevent tumor cells from evading the immune system by enhancing host immunosurveillance antigenicity (reviewed in [88,92,93]). Similar to SIRT1, the mechanisms involved in HDACi function are complex, differ between tumor cell types, and depend on the drug type and exposure time [92,94]. In vitro studies reveal the effects of HDACi are different for each type of cell lineage. However, common among cell types, is the activation of p53 and p21WAF1 and downregulation of cyclins and cell cycle activity following HDACi administration [95].

HDACi vary structurally and are classified in four groups, including hydroxamic acid, benzamides, cyclic peptides and aliphatic acids [90,92,96]. The potential of HDACi to exert anti-cancer effects has been examined in several clinical trials; however, only two HDACi agents have been approved by the Food and Drug Administration to date. SAHA (Vorinostat) and Romidepsin (Istodax) are approved for use in cutaneous T cell lymphoma (CTCL) [96-99]. Current clinical trials are examining HDACi in hematologic malignancies and solid tumors where preliminary results indicate a weaker antitumor activity of HDACi in solid tumors compared to hematological malignancies [92,96,100]. However, many clinical trials involving solid tumors are comprised of patients with advanced disease and with relapsed and metastatic sarcomas (Table 1) (http://www.cancer.gov/clinicaltrials).

The most promising HDACi results involve its synergetic or additive effects with other therapeutic regimens, including radiotherapy, chemotherapy, proteasome inhibitors, death receptor agonists and kinase inhibitors [92,96,100,101]. This is evidenced in HNSCC, where in vitro administration of HDACi exhibits good anticancer properties; however, HDACi shows only limited clinical utility as a single agent [99,102-105]. HDACi is only effective against HNSCC when administered in combination with radiotherapy [106]. For example, Romidepsin shows preclinical activity as a radiosensitizer [101,107].

Although promising, the potential use of HDACi as a new treatment for HNSCC remains poorly explored. Kim et al. [108] examined the pharmacological efficiency of combining the PS-341 protease inhibitor, also known as Bortezomb, with Trichostatin A, an HDAC inhibitor. They found the drugs synergistically induce apoptosis in HNSCC by a mechanism that preserves PS-341-induced endoplasmic reticulum stress. Erl ich et al. analyzed the antitumor effects of AKT and PI3K-mTOR inhibitors delivered as monotherapies, or in combination with HDACi, in models of HNSCC. As a single agent, Vorinostat (HDACi) induces selective cytotoxicity in squamous cell carcinoma, an effect enhanced by PI3K inhibitors that cause persistent AKT inhibition and generation of reactive oxygen species [91]. Further, the use of Panobinostat, a non-selective HDACi resulted in a dose-dependent hyperacetylation of histone H3 and inhibition of tumor proliferation and viability when administered alone. Further, the cytotoxicity effect observed upon administration of Panobinostat was continuously enhanced by co-treatment with PI3K inhibitors. Clinically, the administration of PI3K inhibitors enhanced the cancer cell specific cytotoxicity induced by Panobinostat. There are currently only four registered clinical trials testing HDACi and other therapeutic modalities in HNSCC. These studies involve Valproic acid and platinum-based chemoradiation in advanced HNSCC, Vorinostat in the treatment of advanced staged oropharyngeal squamous cell carcinoma, Capecitabine and Vorinostat in patients with recurrent and/or metastatic HNSCC and CUDC-101 plus cisplatin and radiation in HNSCC (Table 2) (http://www.cancer.gov/clinicaltrials).

Although the use of HDACi has generated great enthusiasm, adverse side effects and resistance have been observed in clinical trials of different cancer types. The main side effects are related to hematologic complications, but nausea, asthenia/fatigue, infections, vomiting, anorexia, diarrhea and pyrexia have been noted. Along with the side effects, the development of tumor resistance to HDACi have been noted although the involved mechanism is far from understood. Additional studies are needed to better understand the mechanisms underlying HDACi action, to explore various combination therapies and to identify which types of cancer will better respond to HDACi treatment [71,100].

The Histone Code of Chromatin Acetylation

Posttranslational chromatin modifications occur through changes to histones. Histone can be modified by acetylation, methylation, and ubiquitination of lysines, methylation of arginines, phosphorylation of serines, sumoylation and ubiquitination, among other recently identified covalent histone modifications [109]. The histone code hypothesizes that histone modifications dynamically influence transcription by recruiting a variety of proteins that activate transcription. Among the numerous histone modifications, we are particularly interested in the effects of chromatin acetylation on tumor behavior. Core histones are composed of a histone-fold domain and an amino-terminal tail domain. Histone acetylation is mediated through interactions of the amino-terminal domain with regulatory proteins that reduce the affinity of the histone to DNA [110]. Therefore, binding efficiency of transcription factors is directly associated with histone-mediated changes in DNA configuration induced by acetylation [111,112].

We are just beginning to elucidate the effects of chromatin acetylation on cellular function. Initial observations by Meegee et al. [113] demonstrated the requirement of lysine to be acetylated in the histone 4 tail domain for cell cycle progression and genomic stability. Further, the acetylation status of histone 4 lysine 16 is implicated in numerous physiological functions across several organisms. In humans, loss of monoacetylation of histone 4 lysine 16 serve as an epigenetic marker for leukemia, breast, lung and colon cancer cells [114] and may be a key event associated with epigenetic silencing of tumor suppressor genes (TSGs) [115]. Interestingly, other histone 4 acetylation residues, such as lysine 5, 8, and 12, are not found in tumors, suggesting that suppression of histone 4 lysine 16 acetylation may be important for cancer prevention. Global patterning of histone acetylation is also observed during the development of the nervous system. Cho et al. demonstrated dynamic histone acetylation expression during neuronal differentiation, as evidenced by increased expression of histone 3 and histone 4 in the cortical plate from rat cerebral cortex (E17) and chick spinal cord (E7) and increased histone 3 in adult mouse dentate gyrus [116]. In the cardiac development system, the HATs p300 and CBP are coactivators that promote the physiological and pathological growth of cardiac myocytes. Similarly, lungs and small intestines require p300 expression during normal development [117]. Collectively, these data suggest a role for acetylated histones in mitosis and differentiation during tissue development and maintenance. In fact, the identification of 3,600 lysine acetylation sites using quantitative mass spectrometry (MS)-based proteomics in 1,750 human proteins indicates that protein modifications mediated by acetylation is far more common than anticipated [118,119]. Although epigenetic modifications result in changes on gene expression independent from alterations on the DNA sequence, tumor epigenome may be influenced by heritable alterations on histones and histones associated transcriptional regulators. Indeed, next-generation sequencing of HNSCC has unveiled several of these
mutations suggesting the differential modulation of epigenetic among normal and cancer cells and with that the existence of a cancer histone code. However, the functional and biological impact of these mutations in HNSCC is largely unknown.

The Next Generation of DNA Sequencing in HNSCC

Recent technological advances in the field of genome sequencing culminated in the development of Next-Generation Sequencing
(NGS). This new technology has substantially improved discovery of single-base changes and the identification of larger structural variants characterized by insertions, deletions, translocations and viral insertions. Several sequencing platforms in use are characterized by amplification of single strands of fragmented DNA followed by sequencing [120].

The work of Agrawal et al. and Stransky et al. comprise the first HNSCC tumor sequencing studies using NGS technology and have provided a novel perspective for this complex disease. This wealth of data derives from 106 sequenced tumors from the head and neck anatomical area, including the oral cavity, oropharynx, hypopharynx, larynx, and sinonasal cavity. Stranksy et al. have identified mutations of well-known genes, including TP53, which is present in 62% of sequenced tumors, and the CDKN2A gene, which occurs in 12% of tumors. In addition to TP53 and CDKN2A, more novel gene mutations were also identified. For example, nonsense, missense and splice site mutations in Notch1 were found in 11% of 74 tumor samples analyzed [121]. Using NGS in 32 head and neck samples, Agrawal et al. found NOTCH1 highly mutated in HNSCC, and approximately 40% of the mutations result in loss of function [122]. Both NGS studies found an inherited genomic difference between HPV-positive and HPV-negative head and neck tumors, with HPV-positive tumors containing less than half the number of mutations evident in HPV-negative tumors [121,122]. This suggests that HPV-positive HNSCC is genetically distinct from HPV-negative HNSCCs, a notion previously implied by their differential responses to chemotherapy and radiotherapy [123-125].

**Novel NGS findings on HNSCC histones Mutations and its Modifiers**

Within the thousands of genes found mutated in HNSCC, NGS have also identified mutations in histones and histone modifiers (Table 3). Of the 74 human head and neck tumor samples sequenced by Stranksy et al. 44 tumors (59.5%) have mutated histones and/or transcriptional coactivators (Figure 1A). NGS analysis also revealed that 12 HNSCC have three or more mutations, and as many as eight mutations were found in a single tumor; 32 tumors harbor one or two mutations in histones and histone associated transcriptional regulators (Figure 1B). The most prevalent mutations are associated with genes encoding the H2A, H2B, H3, and H4 histone families. Similar mutations have been reported in the H3F3A and HIST1H3B genes in pediatric diffuse glioma [126,127], in HIST1H4 in clear cell renal cell carcinoma [128], and in HIST1H4E in human colon and rectal cancer [129]. Interestingly, mutations were also found in HIST1H1B, HIST1H1C, HIST1H1D and HIST1H1E linker histones. Linker histones are characterized by the absence of the histone fold domain and play a key role in organizing maximal nucleosome compaction within the chromatin [130,131] and chromatin binding affinity [132,133], among other functions. Notably, depletion of histone H1 induces aberrant mitotic figures [134], a common histological finding in tumor cells.

The Histone Lysine Demethylases (KDMs) are the second major family of genes mutated in HNSCC. KDMs, a new class of epigenetic enzymes, remove histone marks associated with gene expression and suppression. KDMs play a major role in prostate cancer by suppressing or promoting tumorigenesis [135].

NGS of HNSCC identified additional interesting gene mutations involved in transcription regulation. BCOR, and the BCOL1 encoded corepressor proteins are two examples of transcription regulators capable of selectively inhibit gene expression by interacting with the sequence specific DNA-binding protein BCL6. BCOR interacts with class I and II HDACs and may play an important role in repression. Both BCOR and BCOI1 are found in bone sarcomas and in acute myeloid leukemia [136,137]. The BCOI1 gene also found mutated in HNSCC encodes a corepressor protein that binds to the repressor element-1 silencing transcription factor (REST), but little is known about its involvement in tumor progression.

Transcriptional activators and coactivators are also mutated in HNSCC. Stranksy’s NGS identified mutations in CREBBP and EP300, genes that encode the well-known CREB-binding protein and histone acetyltransferase p300 (p300 HAT) transcriptional activators. CREB-binding protein and histone acetyltransferase p300 coactivators have inherent histone acetyltransferase activity and stabilize proteins at the transcription complex. Mutations in CREBBP and EP300 are associated with the development of Rubinstein-Taybi syndrome and are involved in the development of acute myeloid leukemia. EP300 is mutated in several epithelial tumors, including gastric, breast, cervical, colorectal, pancreatic, and gastric carcinomas [59,60,138-140]. The MYST family of histone acetyltransferases is composed of five human HATs: Tip60 (KAT5), MOZ (MYST3), MORF (MYST4), HBO1 (MYST2) and MOF (MYST1). MYST1, MYST2, and MYST3 are mutated in HNSCC and were recently linked to cancer (reviewed in [141]). A putative role for MYST1 is in modulation of ATM and acetylation of multiple residues of histone 4 [142]. MYST2 is also involved in acetylation of histone 4, but it plays a larger role in DNA replication and is a common site for retroviral integration [143]. MYST2 occurs in a complex with the ING4 and ING5 tumor suppressors, suggesting it plays a suppressive role in carcinogenesis [144]. Similar to MYST2, MYST3 forms a complex with ING5, suggesting that mutations in MYST3 alter the ability of ING5 to regulate p53 function [145]. Lastly, mutation in the EHMT1 histone

| Intervention | Drugs | Condition | Sponsor | Patients enrolled | Phase | Period | Clinical trial number |
|--------------|-------|-----------|---------|------------------|-------|--------|----------------------|
| Vorinostat (SAHA), Cisplatin, Radiation | Stage III or IV of SCC of the oropharynx | Ohio University Comprehensive Cancer Center | 38 | I | 2010-2013 | NCT01064921 |
| Capacitabine (Xeloda), Vorinostat (SAHA) | Recurrent and/or metastatic head and neck cancer, Nasopharyngeal carcinoma, stage III and IV HNSSC and verrucous carcinoma | National (NCI) Cancer Institute | 1445 | II | 2010-2013 | NCT01267240 |
| CUDC-101 Cisplatin, Radiation | Head and neck cancer | Curis, Inc. | 22 | I | 2011-2013 | NCT01384799 |
| Valproic acid, Platinum and Radiation | Locally advanced head and neck SCC (oral cavity and oropharynx) | Intituto do Cancer do Estado de São Paulo | 40 | II | 2012-2014 | NCT01695122 |

SCC: Squamous Cell Carcinoma; HNSSC: Head and Neck Squamous Cell Carcinoma

http://www.cancer.gov/clinicaltrials

**Table 2:** Clinical trials involving HDACi and head and neck squamous cell carcinoma.
| Gene Name | Gene Alias | UniProt | Protein Name | Protein Alias | cDNA Change | Mutation type | Protein Change |
|-----------|------------|---------|--------------|---------------|-------------|---------------|----------------|
| BCOR      | KIAA1575   | Q6W2J9 | BCL-6 corepressor | BCOR          | c.5486G>T   | Nonsense      | p.E1732        |
| BCORL1    |            | Q5H9F3 | BCOR-L1 | BCOR-like protein 1 | c.3989G>A | Missense      | p.R1292Q       |
| CREBBP    | CBP        | Q92793 | CREB-binding protein | ---           | c.4541G>A | Missense      | p.R1446H       |
|           |            |         |            |                | c.4765G>T | Missense      | p.D1521Y       |
|           |            |         |            |                | c.1711C>T | Nonsense      | p.Q503          |
|           |            |         |            |                | c.3053C>T | Missense      | p.T950M         |
|           |            |         |            |                | 9.64G>C   | Missense      | p.A254P         |
| EHMT1     | UHMTASE1, GLP, KIAA1876, KMT1D | Q9H8B1 | EHMT1 | Eu-HMTase1, GLP, GLP1, H3-K9-HMTase 5. | c.1157G>A | Missense      | p.D374N         |
| EHMT2     | BAT8, C6orf30, G9A, KMT1C, NG36 | Q96KQ7 | EHMT2 | Euchromatic histone-lysine N-methyltransferase 2, HLA-B-associated transcript 8, H3-K9-HMTase 3 | c.1155C>T | Missense      | p.S384L         |
| EP300     | P300       | Q90472 | p300 HAT | E1A-associated protein p300 | c.4549G>T | Missense      | p.C1385F       |
|           |            |         |            |                | c.4590G>A | Missense      | p.D1399N       |
|           |            |         |            |                | c.4154G>T | Missense      | p.C1385F       |
| H2BFWT    |           | Q722G1 | Histone H2B type W-T | H2B histone family member W testis-specific | c.451C>T | Missense      | p.R143C         |
| H3F3A     | H3.3A, H3F3, H3.3B | P64243 | H3.3 | --- | c.122C>T | Missense      | p.R3C           |
| H3F3C     | ---        | Q6NXT2 | H3.3C | H3.5 | c.439A>G | Missense      | p.K122E         |
|           |            |         |            |                | c.172A>G | Missense      | p.T33A          |
| H3C5      |            | Q15379 | HD3 | RP3D-2, SMAP45 | c.968G>T | Missense      | p.R301L         |
| H3C4      | KIAA0288   | P56524 | HD4 | --- | c.3452G>T | Missense      | p.G887V         |
| H3C9      | HDAC7, HDAC7B, HDRP, KIAA0744, MTR | Q9UKV0 | HD9 | HD7, HD7b, Histone deacetylase-related protein, MEF2-interacting transcription repressor MTR | c.2643C>A | Missense      | p.H968N         |
| HIST1H1B  | H1F5       | P16401 | H1.5 | H1a, H1b, H1s-3 | c.727G>C | Missense      | p.K225N         |
| HIST1H1E  | H1F4       | P10412 | H1.4 | H1b, H1b-4 | c.146G>A | Missense      | p.G29D          |
| HIST1H2AJ | H2AFE      | Q99878 | H2A type 1-J | H2A/e | c.89G>A | Missense      | p.R30Q          |
|           |            |         |            |                | c.56C>T | Missense      | p.S19F          |
| HIST1H2BF | HIST1H2BC, HIST1H2BE, HIST1H2BG, HIST1H2BI | P62807 | H2B type 1-C/E/F/G/I | H2B/a, H2B/g, H2B/h, H2B/k, H2B/l | c.222G>A | Missense      | p.E77K          |
|           |            |         |            |                | c.152C>G | Missense      | p.P51A          |
| HIST1H2BH | H2BFJ      | Q93079 | H2B type 1-H | H2B/j | c.97>T | Missense      | p.D3E           |
|           |            |         |            |                | c.67C>A | Missense      | p.Q23K          |
| HIST1H3A  | HIST1H3B, HIST1H3C, HIST1H3D, HIST1H3E, HIST1H3F, HIST1H3G, HIST1H3H, HIST1H3I, HIST1H3J | P68431 | H3.1 | H3/a, H3/b, H3/c, H3/d, H3/e, H3/f, H3/h, H3/i, H3/j, H3/k, H3/l | c.125A>G | Missense      | p.Y42C          |
|           |            |         |            |                | c.90G>A | Missense      | p.R27Q          |
|           |            |         |            |                | c.120A>T | Missense      | p.K37M          |
|           |            |         |            |                | c.103A>T | Missense      | p.T33S          |
| HIST1H4B  | HIST1H4A, HIST1H4C, HIST1H4D, HIST1H4E, HIST1H4F, HIST1H4H, HIST1H4I, HIST1H4J, HIST1H4K, HIST1H4L, HIST2H4A, HIST2H4B, HIST4H | P62805 | H4 | --- | c.202C>T | Missense      | p.R68W          |
|           |            |         |            |                | c.77A>T | Missense      | p.N26I          |
|           |            |         |            |                | c.162G>C | Missense      | p.E54D          |
|           |            |         |            |                | c.11G>A | Missense      | p.R4K           |
|           |            |         |            |                | c.262G>T | Missense      | p.V88F          |
|           |            |         |            |                | c.118C>G | Missense      | p.R40G          |
|           |            |         |            |                | c.39A>T | Missense      | p.K13I          |
| HIST2H2AB | Q8UE6 | H2A type 2-B | --- | --- | c.133G>A | Missense      | p.G45R          |
| HIST2H2BE | H2BFQ | Q16778 | H2B type 2-E | H2B/GL105, H2B/lq | c.247G>A | Missense      | p.D69N          |
| KDM3A     | JHDM2A, JMJD1, JMJD1A, KIAA0742, TSGA | Q9Y4C1 | Lysine-specific demethylase, se 3A | Jumonji domain-containing protein 1A | c.3698C>G | Missense      | p.S1124C        |
|           |            |         |            |                | c.4178C>A | Nonsense      | p.S1284         |
| KDM4A     | JHDM3A, JMJD2, JMJD2A, KIAA0677 | O75164 | Lysine-specific demethylase 4A | Jumonji domain-containing protein 2A | c.174C>T | Missense      | p.S3F           |
|           |            |         |            |                | c.503G>A | Missense      | p.E113K         |
|           |            |         |            |                | c.215G0T | Missense      | p.A662S         |
| KDM4B     | JHDM3B, JMJD2B, KIAA0876 | O94953 | Lysine-specific demethylase 4B | Jumonji domain-containing protein 2B | c.1418T>C | Missense      | p.S398P         |
| KDM5A     | JARID1A, RBBP2, RBP2 | P29375 | Lysine-specific demethylase 5A | Jumonji JARID domain-containing protein 1A, RBBP-2 | c.5287G>C | Missense      | p.G1642R        |
|           |            |         |            |                | c.2708G>A | Missense      | p.R782Q         |
| KDM5B     | JARID1B, PLU1, RBBP2H1 | Q9UGL1 | Lysine-specific demethylase 5B | CT31, Histone demethylase JARID1B, Jumonji JARID domain-containing protein 1B, PLU-1, RBBP-2-H1 | c.174A>T | Missense      | p.Y542F         |
|           |            |         |            |                | c.2368G>A | Missense      | p.C751Y         |
that epigenetics equally contributes to cancer development. In addition to technological advances associated with tumor invasion, metastasis and the development of chemoresistance were attributed to genetic instability driven by the accumulation of mutations. Recently, a better understanding of stem cells and developmental biology, in addition to technological advances in the field of full genome sequencing, are providing strong evidence that epigenetics equally contributes to cancer development.

Advances in oncogenomics have also identified mutations in epigenetic associated genes that encode histones and histone modifiers. Synonymous mutations were included. Only missense and nonsense mutations along with frame shift deletions and splice site SNP of histones and histones associated modifiers were included. Synonymous mutations were excluded.

Understanding the link between cancer mutations and epigenetic modulation of transcription will allow design of new therapeutic strategies that may improve the overall survival rate of HNSCC and other cancers. Advances in oncogenomics have also identified mutations in epigenetic associated genes that encode histones and their linkers, proteins associated with the recruitment of DNA-binding proteins, HDAC I and II interacting proteins, corepressor proteins and transcriptional activators and coactivators. Such mutations can dynamically confer tumor advantage over the transcriptional control of pro-survival pathways supporting tumor growth. The complexity of these interactions is evident in the differential effects of HDACi in enhancer and promoter regions may help us understand tumor susceptibility to HAT and HDAC inhibitors and guide the development of new combination therapies.

Understanding the link between cancer mutations and epigenetic modulation of transcription is a potential mechanism involved in tumor progression. The protein encoded by EHMT1 is responsible for directly methylating lysine 9 of histone H3 and is responsible for Kleefstra syndrome (9q34 deletion syndrome) [146].

Conclusions

Increased knowledge of the biology of cancer is reflected in steady improvements in the overall survival rate of various cancers and the continuous reduction in cancer related death in the United States since the 1990s. The latest report on clinical advances in cancer therapy from the American Society of Clinical Oncology (ASCO) highlights the use of multtargeted drugs as the most efficient approach to manage different types of cancer. Emerging therapeutic strategies are using different drugs to target multiple components of common molecular pathways and have shown a positive impact on overall survival in patients with Gastrointestinal Stromal Tumors (GIST), metastatic colorectal carcinomas, neuroblastoma, Anaplastic Large-Cell Lymphoma (ALCL), medullary thyroid carcinoma, breast cancer, and metastatic sarcomas (http://www.cancerprogress.net). Unfortunately, the overall survival rate of HNSCC, one of the most common malignancies worldwide [147], has only marginally improved in the last 50 years despite significant efforts in identify new prognostic markers and understand the molecular signatures involved in tumor progression [148]. Until recently, the molecular circuitry associated with tumor invasion, metastasis and the development of chemoresistance was attributed to genetic instability driven by the accumulation of mutations. Recently, a better understanding of stem cells and developmental biology, in addition to technological advances in the field of full genome sequencing, are providing strong evidence that epigenetics equally contributes to cancer development.

| KDM5C | DXS12722E, JARID1C, SMCX, XE169 | P41229 | Lysine-specific demethylase SC | Histone demethylase JARID1C, Junonji/JARID domain-containing protein 1C, SmcX, protein Xe169 | c.1539G>C | Missense | p.D338H |
|-------|-------------------------------|--------|-------------------------------|-----------------------------------------------|-----------------|-----------|--------|
| MYST1 | KAT8, MOF                     | Q9H7Z6 | MYST-1                         | Lysine acetyltransferase 8, MOZ, YBF2/SAS3, SAS2 and TIP60 protein1, hMOF | c.829C>G | Missense | p.L271V |
| MYST2 | KAT7, HBO1, HBOa              | Q95251 | MYST-2                         | KAT7, Histone acetyltransferase binding to ORC1 Lysine acetyltransferase 7, MOZ, YBF2/SAS3, SAS2 and TIP60 protein 2 | c.179C>T | Missense | p.S18F |
| MYST3 | MOZ, KAT6A, RUNXB2P, ZNF220   | Q92794 | MYST-3                         | KAT6A, MOZ, YBF2/SAS3, SAS2 and TIP60 protein 3 | c.3999G>T | Missense | p.W1152L |
| NAT10 | ALP, KIAA1709                 | Q9H0A0 | N-acetyltransferase 10         | ---                                           | c.1598G>A | Missense | p.A452T |
| PHF21A | BHC80, KIAA1696             | Q6BDS | PHD finger protein 2A           | BHC80a BRAF35-HDAC complex protein BHC80       | c.2553C>G | Missense | p.I643M |
| RCOR1 | KIAA0071, RCOR                | Q9UKL0 | REST corepressor 1             | ---                                           | c.731A>T | Missense | p.K115 |
| RCOR2 | ---                           | Q8IZ40 | REST corepressor 2             | ---                                           | c.659A>T | Missense | p.M122L |
| RCOR3 | KIAA1343                      | Q9P2K3 | REST corepressor 3             | ---                                           | c.206C>A | Missense | p.S55R |
| TNP2  | ---                           | Q0598Z | TP-2                           | ---                                           | c.1016_1017delTT | Frame_Shift_Del | p.L297fs |

Table 3: Histones and histones modifiers found mutated in HNSCC (NGS).

Data represents HNSCC mutations by Stransky N. et al, and Agrawal et al supplemented by UniProt database (http://www.uniprot.org/), and COSMIC (http://cancer.sanger.ac.uk/cancergene/projects/cosmic/). Only missense and nonsense mutations along with frame shift deletions and splice site SNP of histones and histones associated modifiers were included. Synonymous mutations were excluded.
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