PROSPECT
Journal of Cellular Biochemistry 112:394–400 (2011)

Journal of Cellular Biochemistry

Control of RUNX3 by Histone Methyltransferases

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ABSTRACT

Runt-related (RUNX) family proteins function as context-dependent transcription factors during developmental processes such as hematopoiesis, neurogenesis, and osteogenesis. RUNX3 is involved in a variety of physiological processes including neurogenesis, thymopoiesis, and dendritic cell maturation. A large amount of information indicates that RUNX3 may be a tumor suppressor. Recent data suggest that the molecular mechanism responsible for RUNX3 deficiency in numerous cancers is a primarily epigenetic silencing. The present review focuses on the regulation of *RUNX3* gene expression by histone modification, emphasizing histone methylation at the *RUNX3* promoter and inactivation of protein itself. Inactivation of the promoter and protein can be the results of various chemical modifications, including methylation by histone methyltransferase. Inactivation of RUNX3 may contribute to the tumor initiation, progression and pathogenesis in specific microenvironmental contexts. Finally, this review describes the reactivation of *RUNX3* by epigenetic regulatory agents. J. Cell. Biochem. 112: 394–400, 2011. © 2010 Wiley-Liss, Inc.

KEY WORDS: RUNX3; HISTONE ACETYLASES/DEACETYLASES; HISTONE METHYLTRANSFERASES; CANCER; HYPOXIA; INFLAMMATION; SUBCELLULAR LOCALIZATION; METHYLATION; HYPOXIA

The *RUNX* gene family includes three members (RUNX1, RUNX2, and RUNX3). All of these proteins heterodimerize with a common partner, core-binding factor (CBF)-β and are characterized by the highly conserved Runt-homology domain [Ito, 2004]. RUNX1 and RUNX2 are essential for hematopoiesis [de Bruijn and Speck, 2004] and osteogenesis [Stein et al., 2004], and they are genetically deficient in leukemia and bone disorders, respectively. Two different studies of RUNX3 knock-out (KO) mice have revealed that RUNX3 is involved in neurogenesis, the development of the dorsal root ganglion neuron and T lymphocyte differentiation [Li et al., 2002; Fainaru et al., 2004]. There are conflicting data regarding the phenotypes of the gastric epithelium of RUNX3 KO mice with different genetic backgrounds and with different gene targeting constructs [Li et al., 2002; Fainaru et al., 2004], but a lot of evidence suggests that RUNX3 functions as a tumor suppressor of numerous cancers. The present article primarily focuses on the epigenetic mechanisms and potential involvement of histone methyltransferases (HMTs) in *RUNX3* silencing in cancer initiation and progression after a brief review of chromatin remodeling and cancer development. Recent research on the regulating RUNX3 expression by microRNAs under microenvironmental stress conditions is considered. Inactivation of the RUNX3 protein by various chemical modifications in pathological microenvironments is also discussed. Finally, possible therapeutic applications of RUNX3 reexpression and reactivation are presented.

EPIGENETIC CHANGES AND CANCER

Within cells, DNA is packed into chromatin. The nucleosome, the fundamental unit of chromatin, contains 147 base pairs of DNA wound around a core of eight histone proteins. The protein octamer consists of two copies of the H2A, H2B, H3, and H4 proteins, all of which are highly conserved throughout evolution. Four histones have an amino-terminal tail that is lysine rich and positively charged residues of the polypeptide backbone. These amino-terminal tails protrude from the nucleosome and are subject to several types of covalent modification. Post-translational modification of the amino-terminal tails is critical for maintaining chromatin structure and regulating gene expression without altering the DNA sequence (Fig. 1A).

Epigenetic chemical modifications of DNA and histones are important biological phenomena that have been linked to normal cellular differentiation and proper development of an organism. These modifications can regulate gene expression, X chromosome inactivation, genomic imprinting, and chromosomal stability

Grant sponsor: MEST; Grant number: 2010-0026741.
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Received 16 November 2010; Accepted 17 November 2010 • DOI 10.1002/jcb.22969 • © 2010 Wiley-Liss, Inc.
Published online 6 December 2010 in Wiley Online Library (wileyonlinelibrary.com).

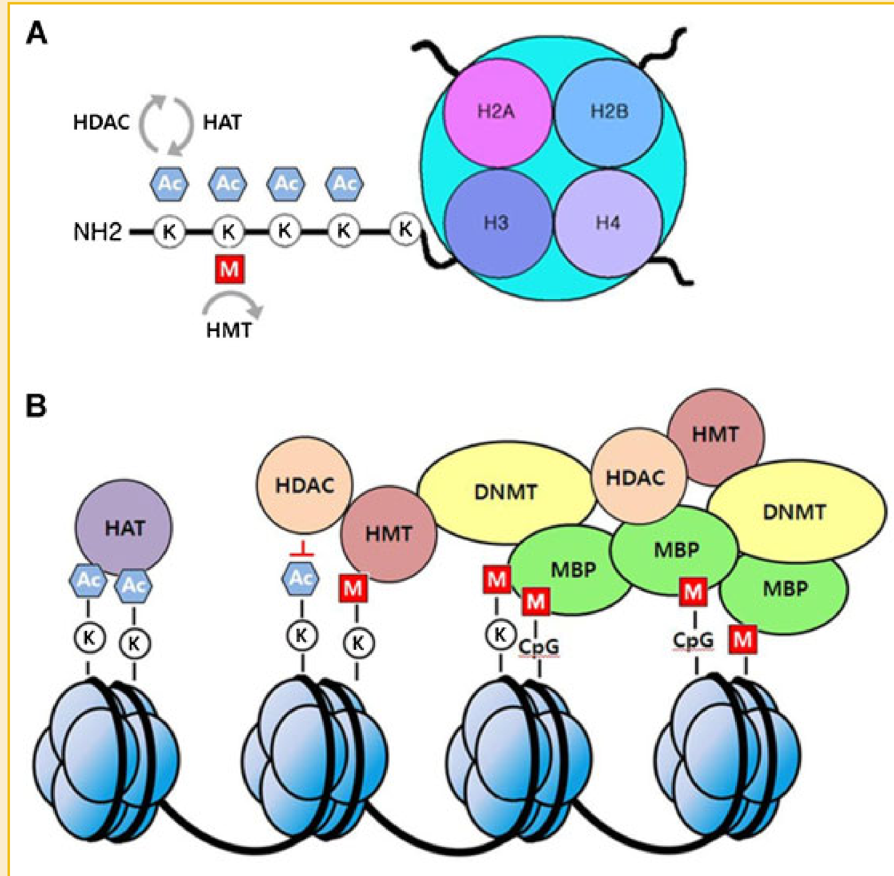

Fig. 1. Post-translational modifications of the core histones and chromatin remodeling that underlie gene silencing in normal and cancer cells. A: Nucleosome structure consisting of two sets of H2A, H2B, H3, and H4. The amino-terminal tails of the histones protrude from the protein core, and the lysine (K) residues of histone H3 are shown as being available for acetylation or deacetylation by histone acetyltransferase (HAT) or histone deacetylase (HDAC) or for methylation by histone methyltransferase (HMT). B: Gene expression is associated with acetylation induced by HAT at lysine (K) residues in the histone tail. In cancer, HDACs deacetylate and/or HMT methylate lysine residues (K) of specific histone tails in a concerted or independent manner. DNA may also be methylated at CpG dinucleotides, mediated by DNA methyltransferases (DNMTs). DNMT or methylated promoters bind methyl-CpG binding proteins (MBDs) that recruit HDACs and HMTs or vice versa.

[Jenuwein and Allis, 2001]. Abnormal alterations to the epigenome have been implicated in a number of human diseases such as cancer, autoimmune diseases, and psychiatric and behavioral disorders [Gibbons, 2005; Ptak and Petronis, 2010]. Extensive studies have demonstrated that DNA methylation of cytosines in CpG dinucleotides of upstream gene promoter regions is an epigenetic marker strongly associated with transcriptional repression and the regulation of chromatin structure. Increasing amounts of evidence have revealed that cytosine methylation often interplay with histone deacetylation [Nan et al., 1997]. It has also been demonstrated that elimination of H3-K9 methylation in *Neurospora* results in the loss of DNA methylation [Tamaru and Selker, 2001]. The reports suggest a functional linkage and sequential interplay between DNA methylation, histone deacetylation, and histone methylation in the formation of the heterochromatic state (Fig. 1B).

Histone lysine acetylation and deacetylation are the best characterized chromatin remodeling mechanisms. Specifically, modification of histone lysine residues by acetylation or deacetylation remodels the structure of nucleosomes. Acetylation of the lysine residues on histone H3 and H4 by histone acetyl transferase (HAT) neutralizes the positive charges of the lysine residues, thereby weakening electrostatic interactions between histones and the phosphate group of DNA. Thus, the acetylation of histones leads to active or open chromatin, which allows various transcription factors access to the promoters of target genes. HATs preferentially acetylate specific histone lysine residues [Roth et al., 2001]. Histone deacetylases (HDACs) remove the acetyl group from lysine, thereby reversing the open chromatin structure and silencing gene expression. Three classes of enzymes can affect the deacetylation of histone proteins; yeast HDAC Rpd3 (Class I), the yeast HDAC Hda (Class II), and the yeast and murine protein Sir2 (Class III) homologs [Zhou et al., 2001]. Only Class III HDACs depend on NAD+ for activity, and they are not sensitive to the HDAC inhibitor trichostatin A (TSA) [Landry et al., 2000]. Interestingly, HAT enzymes target the same transcription factors and glucocorticoid receptor that HDACs do [Gray and Teh, 2001]. It has been shown that RUNX3 interacts with and is acetylated by p300 HAT at lysine residues [Jin et al., 2004]. The acetylation of RUNX3 by p300 HAT, which is required for its transcriptional activity is reversed by HDACs and competes with Smurf1-mediated ubiquitination;

therefore, HDACs stimulate the deacetylation of RUNX3 allowing it to be degraded by the ubiquitin-mediated pathway [Jin et al., 2004].

Methylation of histones at various lysine and arginine residues plays both positive and negative roles in transcription regulation. The methylation of different lysine residues in histones by specific HMTs has also been implicated in the activation and silencing of transcription. Methylation of the histone H3-K4, H3-K36, and H3-K79 is a marker of gene expression and active chromatin, whereas methylation of H3-K9, H3-K27, and H4-K20 is associated with condensed chromatin and the silencing of genes [Gibbons, 2005]. Histone methylation is linked to DNA methylation, because many proteins involved in DNA methylation (such as DNA methyltransferases, DNMTs and methyl-CpG binding proteins, MBPs) also directly interact with HMTs (Fig. 1B).

The first protein shown to possess specific methyltransferase activity toward H3-K9 was the mammalian Suv39h1 enzyme, a homolog of *Schizosaccharomyces pombe* Clr4 and *Drosophila* Su(Var)3.9 [Rea et al., 2000]. This preferential methylation of H3 depends on the SET domain (Suppressor of Variegation, Enhancer of Zeste and Trithorax) [Jenuwein et al., 1998] of Suv39h1, a 130 amino acid domain that is conserved among different species. In addition to Suv39h1, several other methyltransferases have been identified, for example, SET7, SET9, or G9a, which display methylation activities specific to H3-K4, -K9, -K27, -K36, and -K79. Methylation of H3-K9 is one of the most thoroughly studied histone modifications. At least three H3-K9 HMTs have been identified and characterized in mammals. G9a, GLP/EuHMTase I, and ESET/SETDB1 are responsible for the dimethylation of H3-K9 in vivo [Schultz et al., 2002; Tachibana et al., 2005].

Studies in knockout mice for Suv39h1, Suv39h2, and G9a demonstrated that G9a is primarily responsible for monomethylation (Me1) and dimethylation (Me2) of H3-K9, whereas Suv39h1 and Suv39h2 are responsible for direct trimethylation (Me3) of H3-K9. These three states of H3-K9 methylation play a unique role in the structural and functional organization of chromosomes. Me1 and Me2 on H3-K9 reside in euchromatin, while Me2 and Me3 on H3-K9 are found within the two different types of heterochromatin, facultative and constitutive heterochromatin, respectively [Gibbons 2005]. RIZ1 (PRDM2) has also been identified as an HMT that methylates H3-K9 [Derunes et al., 2005]. Arginine residues in histone H3 can be modified to give a monomethyl, asymmetric dimethyl, or symmetric dimethyl states [Gibbons, 2005]. In general, it seems that different degrees of methylation are associated with distinct chromatin regions or transcriptional states [Lee et al., 2005].

Epigenetic changes in conjunction with genetic alterations result in aberrant or total loss of expression of somatic gene products during cancer development. Aberrant patterns of DNA methylation associated with tumor suppressor genes (TSGs) occur early in tumor development and increase progressively, eventually leading to a malignant phenotype [Jones and Baylin, 2007].

Many HDACs exist as components of multiprotein complexes that act as transcriptional corepressors. The recruitment of HDACs to the hypermethylated CpG islands of TSGs by MBPs is strongly associated with cancer development [Deckert and Struhl, 2001]. The regulation of HDAC function under hypoxic conditions was initially demonstrated by Kim et al. [2001]. They suggest that hypoxia increases HDAC activity which leads to a reduction in the expression of two TSGs, *p53* and *von Hippel-Lindau* (*VHL*). The suppression of these two genes resulted in the overexpression of hypoxia inducible factor (HIF)-1α and vascular endothelial growth factor (VEGF), which can be reversed by treatment with the HDAC inhibitor (HDACi) TSA. The report does not demonstrate how HDAC activity is involved in the silencing of *p53* and *VHL* but suggests that HDAC activity might be stimulated by a stress microenvironment to suppress TSG expression [Kim et al., 2001]. HDACs are recruited to TSGs through oncogenic DNA-binding factors independent of DNA methylation [Jones and Baylin, 2007]. It has been demonstrated that the acute myeloid leukemia (AML)-ETO translocation actively suppresses transcription through the aberrant recruitment of HDAC-containing corepressors. It has been also reported that other fusion proteins, such as promyelocytic leukemia protein–retinoic acid receptor can contribute to aberrant CpG-island methylation by recruiting DNMTs and HDACs to aberrant sites [di Croce et al., 2002].

EPIGENETIC SILENCING OF RUNX3 IN CANCER

All RUNX family members are involved in cancer development. To date, the tumor suppressor activity of RUNX3 has been reported for gastric cancer, pancreatic cancer, bladder cancer, colorectal cancer, lung cancer, hepatocellular carcinoma, breast cancer, oral squamous cell carcinoma, prostate cancer, and bile duct cancer [reviewed in Chuang and Ito, 2010].

PROMOTER CpG ISLAND HYPERMETHYLATION AND RUNX3 SILENCING

In approximately 45–60% of human gastric cancer cells, *RUNX3* is not expressed because of hemizygous deletion or hypermethylation of the *RUNX3* promoter [Li et al., 2002]. Inactivation of RUNX3 by hemizygous deletion has been observed in various solid tumors reviewed elsewhere [Ito, 2004; Chuang and Ito, 2010]. In addition to the deletion of the *RUNX3* gene, epigenetic silencing of RUNX3 is one of the most intensively studied subjects in the RUNX research field. Various tissues and cancer cell lines from human patients have *RUNX3* promoter hypermethylation, as identified by methylation-specific PCR or bisulfate genomic sequencing analysis. These tissues and cells include those derived from gastric, bladder, colorectal, breast, lung, pancreatic, brain cancers, and hepatocellular carcinoma [reviewed in Chuang and Ito, 2010]. *RUNX3* hypermethylation in these cancer tissues is strongly associated with poor prognosis and a low patient survival rate. Recently, *RUNX3* hypermethylation status has been used not only as a maker for identifying colorectal tumors [Weisenberger et al., 2006] but also as a risk factor for bladder cancer [Kim et al., 2005]. These reports suggest that RUNX3 methylation occurs early in tumorigenesis and may increase with age.

To date, only three contributors to increase *RUNX3* promoter hypermethylation have been identified. First, *Helicobacter pylori*, a well-known factor involved in gastric cancer, induces *RUNX3* promoter methylation by increasing the production of nitric oxide in macrophages [Katayama et al., 2009]. Second, inflammatory insults such as that caused by lipopolysaccharide (LPS) enhance *RUNX3*

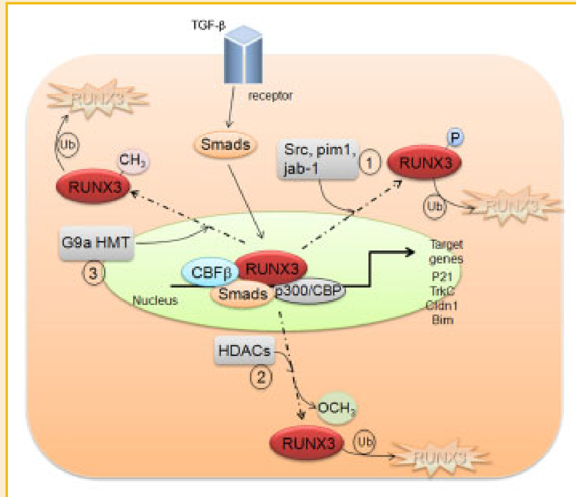

Fig. 2. Inactivation of RUNX3 by aberrant subcellular localization. The RUNX3 protein is activated in the nucleus by the TGF-β signaling pathway, and it activates the transcription of target genes, including p21, TrkC, Claudin 1 (Cldn), and Bim. The phosphorylation by Src, pim-1, and jab-1 induces the cytoplasmic translocation of RUNX3 (1). Deacetylation by HDACs mediates exclusion of RUNX3 from the nucleus (2). Overexpression of G9a HMT also mediates cytoplasmic sequestration of RUNX3 by direct methylation at specific lysine residues in the Runt-homology domain (3). Localized in the cytoplasm, RUNX3 is ubiquitinated and degraded by the Smurf-1-mediated proteasome pathway. These chemical modifications of RUNX3 are involved in the stimulation of nuclear exclusion and protein breakdown.

methylation at the promoter through increased nitric oxide production [Katayama et al., 2009]. These results indicate that inflammation, especially due to *H. pylori* infection, might cause *RUNX3* promoter hypermethylation in gastric cancer. Third, estrogen might be a critical risk factor for *RUNX3* hypermethylation based on a study showing that mammosphere epithelial cells exposed to estrogen exhibited increased hypermethylation of *RUNX3* [Cheng et al., 2008].

HISTONE MODIFICATION AND RUNX3 SILENCING

A recent study showed that enhancer of zeste homolog 2 (EZH2) HMT might be involved in *RUNX3* silencing in gastric, breast, prostate, colon, and pancreatic cancer cell lines by the methylation at H3-K27 [Fujii et al., 2008]. EZH2 indirectly methylates the *RUNX3* promoter CpG island by recruiting of DNMTs [Vire et al., 2006]. Recently, we extended these findings by demonstrating that hypoxia induces epigenetic *RUNX3* silencing through H3K9 methylation and decreased H3 acetylation at the *RUNX3* promoter by G9a and HDAC1, respectively [Lee et al., 2009]. Hypoxia is primarily observed during tumor progression and is associated with inflammatory lesion [Sitkovsky and Lukashev, 2005]. Because inflammation is a putative cause of the initiation of carcinogenesis [Lu et al., 2006] and because hypoxia often occurs in solid tumor masses larger than 1 mm^3, *RUNX3* silencing by hypoxia might be closely associated with initiating carcinogenesis and with tumor progression. *H. pylori* infection and LPS causes *RUNX3* silencing by hypermethylating the *RUNX3* promoter region [Katayama et al., 2009], supporting the hypothesis that *RUNX3* silencing by DNA methylation or histone methylation and deacetylation might be a critical point for the initiation of gastric carcinogenesis. It is commonly accepted that DNA methylation of a target gene promoter by DNMTs mediates the recruitment of HDACs and HMTs [Jones and Baylin, 2007]. There is also a consensus that histone modifications are likely to occur before DNA methylation [Bachman et al., 2003]. However, DNA methylation and histone modifications are coordinated to induce epigenetic changes that silence certain genes [Jones and Baylin, 2007]. Thus, these epigenetic alterations appear to cooperate simultaneously or sequentially to silence *RUNX3*, contributing the initiation or progression of gastric cancer, depending on the microenvironmental cellular context. Under hypoxic conditions, DNA hypermethylation may not occur in the *RUNX3* promoter [Lee et al., 2009], but a number of histone modification enzymes might be activated or inactivated [Rocha, 2005]. Therefore, the modification types and sites in histone tails during *RUNX3* silencing should be investigated under various kinds of microenvironmental stress, including hypoxic conditions.

Another epigenetic mechanism responsible for silencing RUNX3 is microRNAs, which have been recently highlighted as regulators of gene expression at the post-transcriptional level. Loss of microRNA-101, which suppresses EZH2 HMT, results in the overexpression of EZH2 and the induction of H3-K27 trimethylation on the *RUNX3* promoter region in mammary cancer cells [Varambally et al., 2008]. Additionally, it has been suggested that the miR-146, miR-155, miR-21, miR-27a, miR-106-93-25, and miR-221-222 clusters and the miR-200 family might be involved in *H. pylori* infection in gastric carcinogenesis [Belair et al., 2009]. The possible involvement of these microRNAs in *H. pylori*-mediated inflammation and gastric carcinogenesis may implicates them in *RUNX3* silencing. The roles of these miRNAs in silencing RUNX3 should be explored further to evaluate the restoration of *RUNX3* expression for therapeutic approaches. Recently, miR130b became a candidate for silencing *RUNX3* in gastric cancer cells and tissues. Overexpression of miR130b increases cell viability and decreases TGF-β-induced apoptosis, resulting in *RUNX3* protein expression [Lai et al., 2010]. In melanoma tissues, *RUNX3* silencing is linked to miR-532-5p [Kitago et al., 2009]. In summary, *RUNX3* silencing can be regulated by several epigenetic mechanisms, including DNA methylation, histone deacetylation, histone methylation, and some types of microRNAs. Moreover, hypoxia and chronic inflammation mediated by *H. pylori* infection could be causative environmental factors to regulating histone modulation and microRNA expressions to silence *RUNX3* [Katayama et al., 2009], strongly suggesting that the microenvironmental context stimulates aberrant epigenetic silencing of *RUNX3* during cancer progression.

Recent findings demonstrate that G9a, a HMT, methylates nonhistone proteins such as CDYL1, WIZ, ACINUS, and C/EBPβ [Pless et al., 2008; Rathert et al., 2008]. It has also been observed that not only histone methylation on the *RUNX3* promoter but also RUNX3 protein methylation directly induced by G9a at lysine residues in the Runt-homology domain lead to inactivation of RUNX3-mediated transcription and aberrant subcellular localization (our unpublished data, Han et al., submitted). Recently it has been reported that p53 is a target protein for G9a and Glp, its homologous HMT which repress the function of p53 by methylating

K-373 [Huang et al., 2010]. Reptin, a chromatin-remodeling factor, is methylated at K-67 under hypoxic conditions by G9a [Lee et al., 2010]. The G9a-mediated Reptin methylation inhibits HIF-1α-regulated transcription under hypoxic conditions [Lee et al., 2010], suggesting a negative regulation of a subset of hypoxia target gene. However, it is unknown whether RUNX3 methylation by G9a modulates hypoxia-induced responses. G9a induced by a hypoxic microenvironment regulates the chemical status of histones at specific genes, such as *RUNX3* [Lee et al., 2009] and *Mlh1* at the transcriptional level [Chen et al., 2006], and it methylates proteins such as Reptin or RUNX3 to control protein activity. The effects of methylation by G9a on cancer progression might be negative or positive [Lee et al., 2010; our unpublished data, Han et al., submitted]. Fischle et al. [2003] suggested that histone modification enzymes modify nonhistone proteins in amino acid binding cassettes similar to the one in the histone tail region. In particular, the RUNX1, RUNX2, and RUNX3 proteins all have the conserved sequences (SGRGK) found to be modified by HMTs at the arginine and lysine residues in the N-terminus of H4 tail region. These sequences are conserved from *Drosophila* to human. Thus these amino acid sequences might be modified by HMTs; however, it remains unknown which factors induce chemical modification of these short sequences and to what extent these sequences are modified. These conserved motifs could provide discrete information about unknown regulatory mechanisms and functions of RUNX family proteins.

ABERRANT SUBCELLULAR MISLOCALIZATION OF RUNX3 BY HMTS

Another mode of RUNX3 inactivation is the mislocalization of RUNX3. Nuclear expression of RUNX3 is crucial for the expression of target genes. Activation of TGF-β signaling triggers nuclear translocation of endogenous RUNX3 in SNU16 gastric cancer cells [Ito et al., 2005]. Cytoplasmic localization of RUNX3 has been reported in several types of cancer tissues, including colorectal [Ito et al., 2008], gastric [Ito et al., 2005], oral squamous [Gao et al., 2009], and breast cancers [Lau et al., 2006]. In the case of gastric cancer, 38% of cancers demonstrate cytoplasmic expression of RUNX3 [Ito et al., 2005]. Surprisingly, cytoplasmic localization of RUNX3 has been associated with advanced tumor stages in colorectal cancer while nuclear expression has been correlated with better prognosis [Soong et al., 2009]. Several mechanisms for excluding RUNX3 from the nucleus have been suggested. Acetylation of RUNX3 by p300 leads to the stabilization of the RUNX3 protein and induces nuclear localization [Jin et al., 2004]. The phosphorylation of the RUNX3 protein by pim-1 kinase [Kim et al., 2008], Jun-activation domain-binding protein 1 (Jab1/CSN5) [Kim et al., 2009], or c-Src kinase [Goh et al., 2010] is a major cause of cytoplasmic sequestration. Src kinase and Mdm2 E3 ubiquitin ligase have been shown to facilitate cytoplasmic localization of RUNX3 [Chi et al., 2009; Goh et al., 2010]. Mdm2 induces ubiquitin-mediated RUNX3 degradation leading to its cytoplasmic sequestration [Chi et al., 2009]. Recently, we demonstrated that HDAC1 and G9a HMT also mediate RUNX3 exclusion from the nucleus [Lee et al., 2009]. Because HDAC1 counteracts the activity of p300 HAT on nonhistone proteins [Fischle et al., 2003], the acetylation of RUNX3 by p300 can be inhibited by HDAC1, but the role of HDAC1 in the deacetylation of the RUNX3 protein and cytoplasmic localization remains unknown. G9a HMT overexpression also enhances RUNX3 cytosolic expression whereas a knockdown of G9a HMT restores RUNX3 nuclear localization under hypoxic conditions [Lee et al., 2009]. Our further findings show that G9a directly interacts with the RUNX3 protein and methylates specific lysine residues in the Runt-homology domain. This methylation might be a crucial mechanism for aberrant subcellular localization of RUNX3 (unpublished data, Han et al., submitted). In summary, the mechanism by which G9a or HDAC1 mediates RUNX3 cytosolic sequestration remains to be identified.

RUNX3 REACTIVATION BY EPIGENETIC REGULATIONS

The goals of studies of the mechanisms for RUNX3 inactivation in cancer cells, in chronically inflamed cells/tissues or in preneoplastic lesions are the identification of strategies for reactivating RUNX3 to inhibit tumor initiation and progression and the development of biomarkers of tumor initiation and progression. As epigenetic agents, the FDA-approved DNA hypomethylators, 5-azacytidine and 5-aza-2′-deoxycytidine (5-Aza) and HDAC inhibitors such as vorinostat and romidepsin have been used for cancer therapy. A DNMT inhibitor, 5-Aza, reduces the level of H3-K9 methylation but increases H3-K4 methylation in the promoter region of specific TSGs [Nguyen et al., 2002]. Treatment with 5-Aza induces precipitous chromatin remodeling, a reduction in methyl CpG-binding protein 2-binding and H3-K9 methylation, and an increase in histone H3 acetylation at the promoter of the multi-drug resistance gene [El-Osta et al., 2002]. These changes appear to be regional because no changes in the overall levels of modified histones are observed. These results suggest that the inhibition of H3-K9 methylation by 5-Aza treatment is more powerful than the inhibition of cytosine methylation. It is consistently observed that G9a-mediated H3-K9 methylation in the maspin TSG promoter is reduced following 5-Aza treatment [Wozniak et al., 2007]. Therefore, DNMT inhibitors are powerful chemicals to reduce H3-K9 methylation and DNA promoter methylation and to recover RUNX3 activation.

HDACi have pleiotropic effects on numerous cellular targets and have frequently been included in combination therapies with other cytotoxic agents [Beumer and Tawbi, 2010]. As described HDACi also have a strong effect on the reactivation of TSGs under hypoxic conditions [Kim et al., 2001]. In addition to HDAC inhibitory activity, HDACi also have an effect on the acetylation of nonhistone proteins. Thus, HDACi can reverse histone-mediated gene transcriptional repression and can regulate gene activity through protein acetylation [Xu et al., 2007]. We recently demonstrated that TSA dramatically inhibits H3-K9 methylation and increases H3 acetylation, whereas 5-Aza does not induce H3 acetylation but reduces H3-K9 methylation under hypoxic conditions [Lee et al., 2009]. Accumulating evidence indicates that HDACi destabilize HIF-1α which is post-translationally modified by HAT [Beumer and Tawbi,

2010]. It has been well documented that HIF-1α is a critical factor in angiogenesis and metastasis as a result of the induction of its target genes, such as *VEGF* and *CXCR4* [Ellis et al., 2009]. Based on the findings of these reports, it has been suggested that associated with increased tumor size, HDACi more efficiently attenuates the silencing of tumor suppressors, such as *RUNX3*, than DNA hypomethylators do during tumor progression.

This review summarizes the evidence for a critical role of histone modification in *RUNX3* silencing in cancer and other pathological conditions. In association with increasing tumor size and inflammation due to *H. pylori*, the principal underlying mechanisms for silencing *RUNX3* are suggested to be epigenetic changes in the DNA promoter, histone tail modification, and the action of specific microRNAs. *RUNX3* silencing at the transcriptional level and functional inactivation at the protein level in many cancers are highly associated with poor prognosis and may occur in the early stages of tumor initiation. For this reason maintaining RUNX3 expression under microenvironmental stress conditions, either directly or indirectly, has become an attractive target for therapeutic approaches. Other possible mechanisms for regulating RUNX3 should be investigated. Drug discovery to reverse genome-wide silencing of tumor suppressors, including RUNX3, under specific pathological conditions or in cancer cells will be undertaken.

ACKNOWLEDGMENTS

I gratefully acknowledge Prof. Suk-Chul Bae of Chungbuk National University College of Medicine for critical reading of the manuscript and very helpful suggestions. This work was supported by the Mid-career Researcher Program through an NRF grant funded by the MEST (no. 2010-0026741).

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