REVIEW

Clinical and Therapeutic Implications of Histone Acetylation in Breast Cancer

SK Riaz, M Saeed, MFA Malik

ABSTRACT

The contribution of epigenetic changes in triggering breast cancer initiation, promotion, progression and metastasis is an established fact. Altered expression profiling of several genes on DNA is also influenced by histone modifications. In this review, the role of those enzymes regulating histone modifications is discussed. These enzymes are termed as histone acetyltransferases (HATs) and his-tone deacetylases (HDACs). Understanding of the mode of action of these enzymes will be helpful in exploring their antagonistic role on histone DNA complex. In addition to this, the significance of potential histone deacetylases inhibitors (HDIs) as potential cancer therapeutic marker is also discussed.

Keywords: Acetylation, breast cancer, cancer therapeutics

Implicaciones clínicas y terapéuticas de la acetilación de las histonas en el cáncer de mama

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RESUMEN

La contribución de los cambios epigenéticos a desencadenar la iniciación, promoción, progresión y metástasis del cáncer de mama es un hecho establecido. El perfil de expresión alterado de varios genes en el ADN es también influenciado por las modificaciones de las histonas. En este estudio se discute el papel de las enzimas que regulan las modificaciones de las histonas. Estas enzimas son denominadas histonas acetiltransferasas (HATs) e histonas deacetilasas (HDACs). La comprensión del modo de acción de estas enzimas será útil en la exploración de su papel antagónico en el complejo de las histonas de ADN. Además, también se discute la importancia de los potenciales inhibidores de desacetilasas de histonas (IDH) como potencialles marcadores terapéuticos del cáncer.

Palabras claves: Acetilación, cáncer de mama, terapias de cáncer

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INTRODUCTION

Genetic, epigenetic and environmental factors contribute to breast cancer tumorigenesis. According to a World Health Organization (WHO) published report, breast cancer is included among the top five most deadly cancers in women. A cascade of events encompassing chromosomal aberrations, genomic aneuploidies and loss of cell cycle was responsible for carcinogenesis. Both inherited and acquired mutations, either on tumour suppressors or protoncogenes, have significant contribution to cancer development. High penetrance of germline mutations on tumour suppressors (BRCA1, BRCA2 and TP53) in affected cancer patients with positive familial history was observed (1). These mutations were less frequently observed in sporadic cancer patients (2, 3). However, substantial involvement of epigenetic changes including altered chromatin structure, methylation and acetylation profiling has been observed in both inherited and sporadic breast cancer patients (4).

These epigenetic anomalies also regulate tumour suppressor protein expression as observed in sporadic cases of cancers. Effect of these changes on various genes in the context of different types of cancers has also been reported in the literature. Inactivation of von Hippel-Lindau (VHL) in renal (5), AT rich interactive domain 1 A (ARID1A) in ovarian

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and breast (6, 7), isocitrate dehydrogenase 1 (IDH1) in brain (8) and KMT2 in breast cancer (9), 0-6 methylguanine DNA methyltransferase (MGMT) in glioblastoma (10), Werner syndrome RecQ helicase-like (WRN) in cervical cancer (11) and mutL homolog 1 (MLH1) in colorectal and endometrial cancer (12, 13) and mesenchymal related tumours (14) have also been reported. Earlier, epigenetic silencing of BRCA1 *via* promoter methylation has also been reported in breast and ovarian cancer patients (15).

Association of epigenetics with breast cancer with a special focus on histone acetylation and deacetylation has also been explored in this review. Numerous factors responsible for these changes and their relevance to breast cancer initiation and progression have been discussed in subsequent sections.
Chromatin is epigenetically regulated by numerous mechanisms affecting DNA methylation, or histones. Histone modification may include methylation, acetylation and phosphorylation (16, 17). These epigenetic changes lead to chromatin remodelling, resulting in switching on or off of several genes (18, 19).

Histone structure and target sites for acetylation

Chromatin consists of repeated nucleosome units. Each nucleosomal core includes 146 bp DNA wrapped around a histone octamer (20). This octameric structure comprises two sets of four core histone proteins (H2A, H2B, H3 and H4). Histone (H1) is responsible for linking two neighbouring nucleosomal cores. Histone core contains an unstructured N-terminal tail (20–30 residues) and a C-terminal globular domain (70–90 residues). The N-terminal tail is responsible for formation of histone octamer structure, whereas the unstructured portion contains many residues suitable for different covalent modifications like acetylation (21, 22). Multiple lysines, present either on N-terminal tail or in globular domain of histones H3 and H4, are ideal targets for acetylation. These variants of core histones and their interplay with methylation, phosphorylation and ubiquitination and DNA methylation also broaden the scope of epigenetic control (23).

Effect of histone acetylation in cellular process

Differential histone acetylation patterning plays a significant role in regulating transcription, replication, DNA repair and recombination (22). A post-translational acetylation of histone is also closely linked to ageing and other major diseases like cancer, retroviral pathogenesis, cardiovascular diseases and neurodegenerative disorders (24). Abnormal acetylation profiling of histones has an effect on several cellular processes which later proceed to cancer development (22, 25). Both histone acetyltransferases (HATs) and histone deacetylases (HDACs) control transcription by changing the acetylation state of histones and other transcription factors found mostly in the promoter region. Histone acetylation effectively disrupts electrostatic interactions with DNA, leading to decreased chromatin condensation and activation of transcription from that particular region. In addition to this, numerous factors associated with chromatin have shown specific interaction with different domains, like the bromodomain found in HATs and certain adenosine triphosphate (ATP)-dependent chromatin remodelling complexes *eg* the Swi2/Snf2 complex (26, 27). Histone deacetylation represses transcription *via* chromatin condensation.

Histone acetylation: regulation of cell-cycle *versus* metastasis

Tumour cells are usually distinguished by deregulated control on cell-cycle check points. Altered expression of HATs and HDACs do lead to uncontrolled tumour cell proliferation. Earlier, drugs related to HDAC inhibitors were designed to restrict cell proliferation. Trichostatin A (TSA), the first specific HDAC inhibitor, was in fact discovered because it induces cellular differentiation and cell-cycle arrest at G1/S phase of mitosis (28). So far, inhibition is mostly associated with p53-independent induction of $p21^{WAF1/CIP1}$ (29, 30). Hence, exposure of TSA led to reduced cell invasion, metastasis and triggered apoptosis in tumour cells, as observed in gastric cancer (31). In a recent study, the anti-proliferative effect of HDIs like TSA has also been observed in oestrogen receptor (ER) positive tumours. A significant down-regulation of ERα and up-regulation of ERβ at both mRNA and protein levels have been correlated with this inhibitor (32).

Classification of histone acetyltransferases and histone deacetylase

Histone acetyltransferases and HDACs are part of large multisubunit protein complexes (33). Histone acetyltransferases are categorized into three main groups: Gcn5 (general control non-derepressible 5)-related N-acetyltransferases (GNATs), cAMP binding protein (CBP) and MYST (33). The CBP group is unique to metazoans, while members of GNAT and MYST families are present in a variety of hosts ranging from yeast to humans.

Histone deacetylases are responsible for acetyl removal from lysine resides of histone tails and non-histones. These are grouped into four classes where class I includes HDAC 1, 2, 3 and 8. Class I is mainly restricted to the nucleus. Class II comprises HDAC 4, 5, 6, 7, 9 and 10, which play a significant role in regulation of signal-dependent nucleocytoplasmic trafficking. Class III contains seven sirtuins Sir2 (tu), SIRT1–7. Localization of these members either in the nucleus or cytoplasm is still nuclear. Class IV consists of only one member, HDAC11, with a sequence similarity to class I and II members and is mainly localized in the nucleus [Figure] (25).

In the subsequent sections, HATs and HDACs link to breast cancer disease progression is explored.

Histone acetyltransferases family and its role in breast cancer

The (GNAT) General control non-derepressible 5-related N-acetyltransferases family

Several conserved sequence motifs are shared by members of

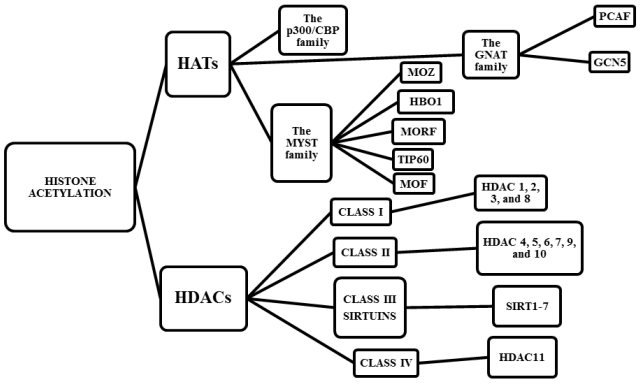

Figure: Classification of histone acetyltransferases (HATs) and histone deacetylases (HDACs).

this family (34). Humans have two Gcn5-like proteins, namely PCAF (p300/CBP-associated factor) and GCN5 involved in regulation of transcription and cell-cycle progression (35, 36). Over-expression of PCAF may affect this differently depending upon the downstream elements activation. Up-regulation of PCAF leads to cell-cycle progression by activating E2F while in others it leads to growth arrest by activating p53. Hence, HAT activity of PCAF has a crucial role in tumour formation (37).

BRCA1 and BRCA2 mutations significantly contribute to breast carcinogenesis (38, 39). Histone acetyltransferases activity of hGCN5 seems to be an essential co-regulator complex in BRCA1-mediated gene regulation (40). Similarly, BRCA2 also interacts with PCAF (41). Interaction among PCAF and BRCA2 is also influenced by other proteins including a co-activator called GRIP1 *via* activation domain (AD1 and AD2) and androgen receptor (AR) cooperation (42). A mechanistic understanding of interactions among BRCA1/BRCA2 and GCN5/PCAF still remain to be elucidated in relation to breast cancer progression.

The p300/CBP family

Members involved in this family are homologous co-activators of transcriptional machinery. Expression of these proteins also regulates cell growth, transformation, differentiation and apoptosis. cAMP-binding protein was discovered as a co-activators of transcriptional machinery. Expression of these proteins also regulates cell growth, transformation, differentiation and apoptosis. cAMP-binding protein was discovered as a co-activator of CREB, a transcription factor (43), while p300 was isolated from adenoviral oncogenic protein E1A as its target (44). Recombinant CBP/p300 has been shown to acetylate all four histone molecules with a relatively reduced substrate specificity when compared with other HATs. Apart from histones, CBP/p300 acetylates a wide variety of transcription regulatory proteins like tumour suppressor p53 (45). Anti-proliferative effects of p53 are mediated by C-terminal domain acetylation which is critical for ubiquitination and genome stability (46).

The MYST family

In humans, the presence of five members belonging to this family has been observed. It includes HBO1 (HATbound to Orc1), MOZ (monocytic leukaemia zinc finger protein), MORF (MOZ-related factor), MOF (males absent on the first) and Tip60 [Tat-interacting protein of 60kDa] (37). HBO1 acts as a co-regulator for many nuclear hormone receptors interacting with the human origin recognition complex (47). Up-regulation of HBO1 in various subsets of primary cancers has also been observed (48). Over-expression of HBO1 is observed in testis, breast, ovary, stomach, bladder and oesophageal cancers (49). Association of MOZ and MORF expression with leukaemia is already established (50, 51). However, their association with breast cancer progression has yet to be explored. Activity of MOF in cultured mammalian cells is found in response to DNA damage when studied in breast cancer (52, 53). Involvement of Tip60 in DNA repair and apoptosis *via* modulating Myc and p53 has also been published (54–56). Hence, MYST role in inducing tumour suppression or aggravation is also influenced by molecular cross-road interactions and signalling pathways.

Histone deacetylation and its role in breast cancer

Histone deacetylase activity established tumorigenesis by deacetylation of histone H4 at lysine 16 (4). Histone deacetylase abnormal expression has also been associated with cancer and it has significance as a therapeutic target. A brief outline of HDACs has also been mentioned in the Table.

Table: Classification of histone deacetylases

HDAC	Types	Localization	Functions	Expression
Class I	HDAC 1, 2, 3 HDAC 8	Nucleus Cytosol	Mediate gene repression in response to DNA damage, cell proliferation, cell cycle control and apoptosis	Over-expression
Class IIa	HDAC 4, 5, 7	Tissue specific	Differentiation and development of vascular, cardiac, immune and nervous systems	Over-expression and mutation
Class IIb	HDAC 6 HDAC 10	Cytoplasm Nucleus and cytoplasm	Development of breast stem cells Not known	Over-expression
Class III (sirtuins)	SIRT 1, 6, 7 SIRT 2 SIRT 3, 4, 5	Nucleus Cytosol Mitochondria	DNA repair, oxidative stress, metabolism and ageing	Variable
Class IV	HDAC 11	Nucleus	Development of oligodendrocyte and immune system response	Not known

Class I HDACs

Class I includes HDAC 1, 2, 3 and 8, which are mainly restricted to the nucleus. Expression of Class I HDACs is universal in all tissues. They apply a powerful catalytic effect on histone lysine residues. Histone deacetylases and HDAC2 are analogous in structure and have functional significance in response to DNA damage, cell proliferation, cell cycle control and apoptosis, therefore, they play a vital role in physiology and development of an organism (57, 58). Furthermore, HDAC8 is primarily localized in the cytosol and hence its expression is critical for differentiation of smooth muscle cells (59). Class I HDACs contain extremely conserved deacetylase domain with short amino- and carboxy-terminal (60). Class I HDACs mediate gene repression as a component of a multi-protein complex. The Sin3, CoREST (corepressor of RE1 silencing transcription factor) and Mi-2/NurD (nucleosome remodelling deacetylase) complexes contain HDAC1 and 2 as their catalytic subunit, while HDAC3 is generally employed on targets by the N-CoR (nuclear receptor corepressor)/SMRT (silencing mediator for retinoid and thyroid receptors) complex. So far, there is no evidence of existence of HDAC8 as being a part of protein complex (60).

Deregulation of the Class I subfamily members of HDACs is found invariably in several cancers. Over-expression of HDAC1 has been investigated in breast, gastric, pancreatic, lung, prostate and hepatocellular carcinomas which in most cases is linked with poor prognosis (61–63). Histon deacetylases and HDAC3 expression can also serve as prognostic marker in breast cancer because of its correlation with ER and progesterone receptor (PR) expression (64). Using tissue microarray analysis for malignant mesenchymal tumours, levels of HDAC2 were found to be elevated in comparison to HDAC1 (65). Over-expression of class I HDAC is also linked with poor prognosis. A thorough insight for HDAC expression profiling with breast cancer progression is still an area that require further investigation.

Class II HDACs

Class II has further been subdivided into two subsets.

Class IIa HDACs

Class IIa has tissue-specific expression. Members of Class IIa are HDAC 4, 5 and 7. Class IIa HDACs have repressive impact over the vascular system the immune system and the brain. Members of Class IIa HDACs contain a long regulatory N-terminal domain flanking the conserved deacetylase domain which controls tissue-specific expression mediated by co-repressors (66). Phosphorylation of serine residues in the amino terminal determines signal localization inside the cell. Catalytic activity of histone deacetylase domain still needs to be explored. Class IIa HDACs act as a component of the repressor complex SMRT/N-CoR (67).

Histone deacetylases 4 expression in breast cancer was higher in comparison to bladder, renal and colorectal cancer (68). Expression dysregulation or HDAC4 mutation is also associated with breast cancer (69). HDAC5, in association with TBX3, represses p14 in breast cancer cells and stimulates cell proliferation (70). HDAC5 also participates in the progression of replication fork in cancer cells, hence maintaining the structure of heterochromatin (71). HDAC7 plays a part in cell growth by repressing reprimo, a tumour suppressor gene and cell cycle inhibitor, in association of ERα (72). HDAC7 interacts with ACTN4 in breast cancer cells to promote cancer cell proliferation by enhancing transcriptional activity of ERα (73).

Class IIb HDACs

Two members of the class IIb subfamily are HDAC6 and HDAC10. HDAC6 is found mainly in the cytoplasm. It consists of a carboxy-terminus zinc finger and two deacetylase domains. Its major target in cytoplasm is α-tubulin. HDAC10 is localized in both the nucleus and cytoplasm and it also has an extra deacetylase domain. The substrates specific for HDAC10 still remain unidentified (74).

Expression of HDAC6 has been found elevated in tumorigenesis (75). Expression of HDAC6 in breast cancer is also associated with enhanced survival. HDAC6 level is significantly correlated with tumour grade, size and positive ER and PR. HDAC6 may serve as a prognostic marker for breast cancer development as well as a predictive indicator of sensitivity to hormonal therapy (76). However, no significant correlation of HDAC6 expression with breast cancer tissues has been established (77). HDAC6 has been reported to affect the development of breast stem cells by deacetylating chaperone Hsp90 which results in inhibition of activation of steroid receptor-mediated transcription (78). Involvement of HDAC10 has not yet been established in cancer initiation or progression.

Class III HDACs – sirtuins

A diversified involvement of sirtuins in a variety of biological functions related to DNA repair, oxidative stress, metabolism and ageing has been observed. Sirtuins are found in different compartments of cell like SIRT1, SIRT6 and SIRT7 and are mostly present in the nucleus. SIRT2 is localized in the cytosol and SIRT3, 4 and 5 mainly exist in the mitochondria (79). Current studies suggest an association of sirtuins to cancer. However, similar to other HDACs, sirtuins also have a tumour suppressor as well as pro-oncogenic function in cancer. Like other HDACs, abnormal expression of many sirtuins is present in many types of cancer. Expression of SIRT3 and SIRT7 is high in breast cancer, while SIRT3 demonstrates variable expression in different types of breast cancer, in which it can be up-regulated or down-regulated (80, 81).

Class IV HDACs

Histone deacetylase 11 is presently the only member in Class IV HDAC. HDAC11 has conserved residues in the catalytic domain which is common in Cass I and Class II HDACs (82). HDAC11 expression is high in the brain, kidney, testis, skel-

etal muscle and heart but little is known about its function. It has been linked with the development of oligodendrocyte and immune system response (83, 84). Limited studies have proven the potential function of HDAC11 in breast carcinogenesis.

Histone deacetylase inhibitors: New era of therapeutics

Aberrant HDAC activity has been documented in a variety of tumour types and so HDAC inhibitors are being developed as anticancer therapeutics. Currently available HDAC inhibitors target a variety of HDAC enzymes with Class 1 (HDAC 1, 2, 3 and 8), Class 2 (HDAC 4–7 and 9–10) and Class 4 (HDAC 11) activity. Modest clinical benefits were previously reported with relatively weak HDAC inhibitors such as valproic acid and phenylbutyrate in advanced solid tumours or haematologic malignancies (85). More potent HDAC inhibitors including both class-specific inhibitors (entinostat and romidepsin) and pan HDAC inhibitors (vorinostat, belinostat and panobinostat) have been developed recently.

Research which has been conducted up to now supports the exploration of HDIs in breast cancer therapy. Vorinostat, an HDI, stimulates differentiation or growth arrests in various human cancers including breast carcinoma (86, 87). Vorinostat also tends to reduce tumour prevalence in rat mammary tumour development induced by 40 % NMU (88). In vitro investigations provided proof signifying that vorinostat precludes clonogenic growth of ER-negative and ER-positive breast cancer cell lines by invigorating G1 and G2/M cell cycle, arrest followed by apoptosis (89). Vorinostat administered in low concentrations is also associated with cell deposition mainly in G1 phase of the cell cycle, and elevated concentrations of vorinostat initiate growth arrest primarily in G2/M phase of the cell cycle (86). Histone deacetylase inhibitor to lessen transcriptional repression in preclinical breast cancer models has also been examined. Accumulation of acetylated H3 and H4 histone tails in combination with re-expression of a functional ER in ER-negative breast cancer cell lines has been observed with a novel HDAC inhibitor, scriptaid (90). Treatment of ER-negative breast cancer cell lines with vorinostat is associated with reactivation of silenced ER, as well as downregulation of DNMT1 and EGFR protein expression (91). Epigenetically reactivated exposure of MDA-MB-231 breast cancer cells to tamoxifen sensitivity restored ER-negativity following treatment with both HDAC (TSA) and DNMT inhibitors [DAC] (92). Entinostat has been shown to induce not only re-expression of ERα, but also the androgen receptor and the aromatase enzyme (CYP19) both in vitro and triple-negative breast cancer xenografts (93). In addition, the combination of entinostat and letrozole resulted in a significant and durable reduction in the xenograft tumour volume when compared to treatment with either agent alone. These experiments have provided the strong rationale for combining epigenetic modifiers with hormonal therapy in breast cancer clinical trials (94). Several studies indicate a strategy which combines both HDAC and DNMT inhibitors for efficacious silencing of genes and restoration of response to tamoxifen and aromatase inhibitors (90, 93, 95).

Association of RARβ in inducing tumour suppression of epithelial cells has been observed. RARβ reactivation also, along with both HDAC and DNMT inhibitors, significantly increases tumour suppression (96). Clinical studies investigating the retinoids in various breast cancer populations to date have yielded disappointing results, but perhaps the lack of efficacy observed relates to the fact that RARβ expression was not evaluated in the majority of these studies (97). Pretreatment of various tumour cell lines with HDAC inhibitors increases the cytotoxicity of chemotherapy. Administering the HDAC inhibitor after chemotherapy did not achieve the same results, suggesting that pretreatment with these agents may open the chromatin structure and thus facilitate an enhanced anticancer effect of chemotherapy drugs that target DNA (98). In breast cancer cell lines with amplification and over-expression of HER2, HDAC inhibitor use depleted HER2 by attenuation of its mRNA levels and promotion of proteosomal degradation. HDAC inhibition was also enhanced because of apoptosis induced by trastuzumab, docetaxel, epothilone B and gemcitabine (99). HDAC inhibitors also significantly enhance trastuzumab-induced growth inhibition in trastuzumab-sensitive, HER2-over-expressing breast cancer cells, providing a strong rationale for clinical studies with this combination in patients with HER2-positive disease (100, 101). Hence, further studies in this regard will aid in the development of a potential therapeutic remedy where breast cancer research is concerned.

CONCLUSION

Epigenetics research has revolutionized cancer diagnosis and therapeutics. Acetylation pattern of chromatin is a milestone but it still remains to be completed after sequencing of the whole epigenome. Inhibitors of HDAC do overcome therapeutic challenges in breast cancer research. Deciphering these challenges will unleash novel scientific perspectives in the HDAC inhibitors, a future prospect for personalized medicine.

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REFERENCES

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; **144:** 646–74.
2. Malik FA, Ashraf S, Kayani MA, Jiang WG, Mir A, Ansar M et al. Contribution of BRCA1 germline mutation in patients with sporadic breast cancer. Int Semin Surg Oncol (now named World J Surg Oncol) 2008; **5:** 21.
3. Fanale D, Amodeo V, Corsini LR, Rizzo S, Bazan V, Russo A. Breast cancer genome- wide association studies: there is strength in numbers. Oncogene 2012; **31:** 2121–8.
4. Esteller M. Epigenetics in cancer. N Engl J Med 2008; 358: 1148–59.
5. Herman JG, Latif F, Weng Y, Lerman MI, Zbar B, Liu S et al. Silencing of the VHL tumour-suppressor gene by DNA methylation in renal carcinoma. Proc Natl Acad Sci USA 1994; **91:** 9700–4.
6. Jones S, Li M, Parsons DW, Zhang X, Wesseling J, Kristel P et al. Somatic mutations in the chromatin remodeling gene ARID1A occur in sev-

eral tumour types. Hum Mutat 2012; **33:** 100–3.
7. Jones S, Wang TL, Shih IM, Mao TL, Nakayama K, Roden R et al. Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma. Science 2010; **330:** 228–31.
8. Noushmehr H, Weisenberger DJ, Diefes K, Phillips HS, Pujara K, BP et al. Cancer Genome Atlas Research Network. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. Cancer Cell 2010; **17:** 510–22.
9. Stephens PJ, Tarpey PS, Davies H, Van Loo P, Greenman C, Wedge DC; Oslo Breast Cancer Consortium (OSBREAC) et al. The landscape of cancer genes and mutational processes in breast cancer. Nature 2012; **486:** 400–4.
10. Esteller M, Garcia-Foncillas J, Andion E, Goodman SN, Hidalgo OF, Vanaclocha V et al. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. N Engl J Med 2000; 343: 1350–4. Erratum. N Engl J Med 2000; **343:** 1740.
11. Masuda K, Banno K, Yanokura M, Tsuji K, Kobayashi Y, Kisu I et al. Association of epigenetic inactivation of the WRN gene with anticancer drug sensitivity in cervical cancer cells. Oncol Rep 2012; **28:** 1146–52.
12. Esteller M, Levine R, Baylin SB, Ellenson LH, Herman JG. MLH1 promoter hypermethylation is associated with the microsatellite instability phenotype in sporadic endometrial carcinomas. Oncogene 1998; **17:** 2413–7.
13. Sinicrope FA, Sargent DJ. Molecular pathways: microsatellite instability in colorectal cancer: prognostic, predictive, and therapeutic implications. Clin Cancer Res 2012; **18:** 1506–12.
14. Teng IW, Hou PC, Lee KD, Chu PY, Yeh KT, Jin VX et al. Targeted methylation of two tumour suppressor genes is sufficient to transform mesenchymal stem cells into cancer stem/initiating cells. Cancer Res 2011; 71: 4653–63.
15. Rice JC, Ozcelik H, Maxeiner P, Andrulis I, Futscher BW. Methylation of the BRCA1 promoter is associated with decreased BRCA1 mRNA levels in clinical breast cancer specimens. Carcinogenesis 2000; **21:** 1761–5.
16. Yang XJ, Seto E. HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention. Oncogene 2007; **26:** 5310–18.
17. Kim GW, Yang XJ. Comprehensive lysine acetylomes emerging from bacteria to humans. Trends Biochem Sci 2011; **36:** 211–20.
18. Baylin SB, Herman JG. DNA hypermethylation in tumourigenesis: epigenetics joins genetics. Trends Genet 2000; **16:** 168–74.
19. Baylin SB, Jones PA. A decade of exploring the cancer epigenome – bio logical and translational implications. Nat Rev Cancer 2011; 11: 726–34.
20. Khorasanizadeh S. The nucleosome: from genomic organization to genomic regulation. Cell 2004; **116:** 259–72.
21. Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 resolution. Nature 1997; **389:** 251–60.
22. Kouzarides T. Chromatin modifications and their function. Cell 2007; **128:** 693–705.
23. Berger SL. The complex language of chromatin regulation during transcription. Nature 2007; **447:** 407–12.
24. Haberland M, Montgomery RL, Olson EN. The many roles of histone deacetylases in development and physiology: implications for disease and therapy. Nat Rev Genet 2009; **10:** 32–42.
25. Glozak MA, Seto E. Histone deacetylases and cancer. Oncogene 2007; **26:** 5420–32.
26. Hassan AH, Prochasson P, Neely KE, Galasinski SC, Chandy M, Carozza MJ et al. Function and selectivity of bromodomains in anchoring chromatin-modifying complexes to promoter nucleosomes. Cell 2002; **111:** 369–79.
27. Mujtaba S, Zeng L, Zhou MM. Structure and acetyl-lysine recognition of the bromodomain. Oncogene 2007; 26: 5521–7.
28. Yoshida M, Kijima M, Akita M, Beppu T. Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. J Biol Chem 1990; 265: 17174–9.
29. Vrana JA, Decker RH, Johnson CR, Wang Z, Jarvis WD, Richon VM et al. Induction of apoptosis in U937 human leukemia cells by suberoy lanilidehydroxamic acid (SAHA) proceeds through pathways that are regulated by Bcl-2/Bcl-XL, c-Jun, and p21CIP1, but independent of p53. Oncogene 1999; **18:** 7016–25.
30. Richon VM, Sandhoff TW, Rifkind RA, Marks PA. Histone deacetylase inhibitor selectively induces p21WAF1 expression and gene-associated histone acetylation. Proc Natl Acad Sci USA 2000; **97:** 10014–9.
31. Yasui W, Oue N, Ono S, Mitani Y, Ito R, Nakayam AH. Histone acetylation and gastrointestinal carcinogenesis. Ann N Y Acad Sci 2003; **983:** 220–31.
32. Duong V, Bret C, Altucci L, Mai A, Duraffourd C, Loubersac J et al. Specific activity of class II histone deacetylases in human breast cancer cells. Mol Cancer Res Dec 2008; **6:** 1908–19.
33. Allis CD, Berger SL, Cote J, Dent S, Jenuwien T, Kouzarides T et al. New nomenclature for chromatin-modifying enzymes. Cell 2007; **131:** 633–6.
34. Neuwald AF, Landsman D. GCN5-related histone N-acetyltransferases belong to a diverse superfamily that includes the yeast SPT10 protein. Treds Biochem Sci 1997; **22:** 154–5.
35. Yang XJ, Ogryzko VV, Nishikawa J, Howard BH, Nakatani Y. A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. Nature 1996; **382:** 319–24.
36. Ogryzko VV, Kotani T, Zhang X, Schiltz RL, Howard T, Yang XJ et al. Histone-like TAFs within the PCAF histone acetylase complex. Cell 1998; **94:** 35–44.
37. Timmermann S, Lehrmann H, Polesskaya A, Harel-Bellan A. Histone acetylation and disease. Cell Mol Life Sci 2001; **58:** 728–36.
38. Lancaster JM, Wooster R, Mangion J, Phelan CM, Cochran C, Gumbs C et al. BRCA2 mutations in primary breast and ovarian cancers. Nat Genet 1996; **13:** 238–40.
39. Boulton SJ. Cellular functions of the BRCA tumour-suppressor proteins. Biochem Soc Trans 2006; **34:** 633–45.
40. Oishi H, Kitagawa H, Wada O, Takezawa S, Tora L, Kouzu-Fujita M et al. An hGCN5/TRRAP histone acetyltransferase complex co-activates BRCA1 transactivation function through histone modification. J Biol Chem 2006; **281:** 20–6.
41. Fuks F, Milner J, Kouzarides T. BRCA2 associates with acetyltransferase activity when bound to PCAF. Oncogene 1998; **17:** 2531–4.
42. Shin S, Verma IM. BRCA2 cooperates with histone acetyltransferases in androgen receptor-mediated transcription. Proc Natl Acad Sci USA 2003; **100:** 7201–6.
43. Chrivia JC, Kwok RP, Lamb N, Hagiwara M, Montminy MR, Goodman RH. Phosphorylated CREB binds specifically to the nuclear protein CBP. Nature 1993; **365:** 855–9.
44. Eckner R, Ewen ME, Newsome D, Gerdes M, DeCaprio JA, Lawrence JB et al. Molecular cloning and functional analysis of the adenovirus E1A-associated 300-kD protein (p300) reveals a protein with properties of a transcriptional adaptor. Genes Dev 1994; **8:** 869–84.
45. Sterner DE, Berger SL. Acetylation of histones and transcription-related factors. Microbiol Mol Biol Rev 2000; **64:** 435–59.
46. Gu W, Roeder R. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell 1997; **90:** 595–606.
47. Iizuka M, Stillman B. Histone acetyltransferase HBO1 interacts with the ORC1 subunit of the human initiator protein. J Biol Chem 1999; **274:** 23027–34.
48. Georgiakaki M, Chabbert-Buffet N, Dasen B, Meduri G, Wenk S, Rajhi L et al. Ligand-controlled interaction of histone acetyltransferase binding to ORC-1 (HBO1) with the N-terminal transactivating domain of pro gesterone receptor induces steroid receptor coactivator 1-dependent coactivation of transcription. Mol Endocrinol 2006; **20:** 2122–40.
49. Iizuka M, Takahashi Y, Mizzen CA, Cook RG, Fujita M, Allis CD et al. Histone acetyltransferase Hbo1: catalytic activity, cellular abundance, and links to primary cancers. Gene 2009; **436:** 108–14.
50. Champagne N, Bertos NR, Pelletier N, Wang AH, Vezmar M, Yang Y et al. Identification of a human histone acetyltransferase related to monocytic leukemia zinc finger protein. J Biol Chem 1999; **274:** 28528–36.
51. Avvakumov N, Cote J. The MYST family of histone acetyltransferases and their intimate links to cancer. Oncogene 2007; **26:** 5395–407.
52. Gupta A, Guerin-Peyrou TG, Sharma GG, Park C, Agarwal M, Ganju RK et al. The mammalian ortholog of Drosophila MOF that acetylates histone H4 lysine 16 is essential for embryogenesis and oncogenesis.

Mol Cell Biol 2008; **28:** 397–409.
53. Pfister S, Rea S, Taipale M, Mendrzy KF, Straub B, Ittrich C et al. The histone acetyltransferase hMOF is frequently downregulated in primary breast carcinoma and medulloblastoma and constitutes a bio marker for clinical outcome in medulloblastoma. Int J Cancer 2008; **122:** 1207–13.
54. Ikura T, Ogryzko VV, Grigoriev M, Groisman R, Wang J, Horikoshi M et al. Involvement of the TIP60 histone acetylase complex in DNA repair and apoptosis. Cell 2000; **102:** 463–73.
55. Squatrito M, Gorrini C, Amati B. Tip60 in DNA damage response and growth control: many tricks in one HAT. Trends Cell Biol 2006; **16:** 433–42.
56. MeL L, Vidal F, Gallardo D, Diaz-Fuertes M, Rojo F, Cuatrecasas M et al. New p53 related genes in human tumours: significant downregulation in colon and lung carcinomas. Oncol Rep 2006; **16:** 603–8.
57. Segre CV, Chiocca S. Regulating the regulators: the post-translational code of class I HDAC1 and HDAC2. J Biomed Biotechnol 2011; **2011:** 690848.
58. Reichert N, Choukrallah MA, Matthias P. Multiple roles of class I HDACs in proliferation, differentiation, and development. Cell Mol Life Sci 2012; **69:** 2173–87.
59. Waltregny D, Glenisson W, Tran SL, North BJ, Verdin E, Colige A et al. Histone deacetylase HDAC8 associates with smooth muscle alpha-actin and is essential for smooth muscle cell contractility. FASEB J 2005; **19:** 966–8.
60. Yang XJ, Seto E. The Rpd3/Hda1 family of lysine deacetylases: from bacteria and yeast to mice and men. Nat Rev Mol Cell Biol 2008; 9: 206–18.
61. Zhang Z, Yamashita H, Toyama T, Sugiura H, Ando Y, Mita K et al. Quantitation of HDAC1 mRNA expression in invasive carcinoma of the breast. Breast Cancer Res Treat 2005; **94:** 11–6.
62. Choi JH, Song YS, Yoon JS, Song KW, Lee YY. Enhancer of zeste homolog 2 expression is associated with tumour cell proliferation and metastasis in gastric cancer. Apmis 2010; **118:** 196–202.
63. Minamiya Y, Ono T, Saito H, Takahashi N, Ito M, Mitsui M et al. Expression of histone deacetylase 1 correlates with a poor prognosis in patients with adenocarcinoma of the lung. Lung Cancer 2011; **74:** 300–4.
64. Krusche CA, Wulfing P, Kersting C, Vloet A, Bocker W, Kiesel L et al. Histone deacetylase-1 and -3 protein expression in human breast cancer: a tissue microarray analysis. Breast Cancer Res Treat 2005; **90:** 15–23.
65. Pacheco M, Nielsen TO. Histone deacetylase 1 and 2 in mesenchymal tumours. Mod Pathol 2012; **25:** 222–30.
66. Parra M, Verdin E. Regulatory signal transduction pathways for class IIa histone deacetylases. Curr Opin Pharmacol 2010; **10:** 454–60.
67. Fischle W, Dequiedt F, Hendzel MJ, Guenther MG, Lazar MA, Voelter W et al. Enzymatic activity associated with class II HDACs is dependent on a multiprotein complex containing HDAC3 and SMRT/N-CoR. Mol Cell 2002; **9:** 45–57.
68. Ozdag H, Teschendorff AE, Ahmed AA, Hyland SJ, Blenkiron C, Bo brow L et al. Differential expression of selected histone modifier genes in human solid cancers. BMC Genomics 2006; **7:** 90.
69. Sjoblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD et al. The consensus coding sequences of human breast and colorectal cancers. Science 2006; **314:** 268–74.
70. Yarosh W, Barrientos T, Esmailpour T, Lin L, Carpenter PM, Osann K et al. TBX3 is overexpressed in breast cancer and represses p14 ARF by interacting with histone deacetylases. Cancer Res 2008; 68: 693–9.
71. Peixoto P, Castronovo V, Matheus N, Polese C, Peulen O, Gonzalez A et al. HDAC5 is required for maintenance of pericentric heterochromatin, and controls cell-cycle progression and survival of human cancer cells. Cell Death Differ 2012; **19:** 1239–52.
72. Malik S, Jiang S, Garee JP, Verdin E, Lee AV, O'Malley BW et al. Histone deacetylase 7 and FoxA1 in oestrogen-mediated repression of RPRM. Mol Cell Biol 2010; **30:** 399–412.
73. Khurana S, Chakraborty S, Cheng X, Su YT, Kao HY. The actin-binding protein, actinin alpha 4 (ACTN4), is a nuclear receptor coactivator that promotes proliferation of MCF-7 breast cancer cells. J Biol Chem 2010; **286:** 1850–9.
74. Hubbert C, Guardiola A, Shao R, Kawaguchi Y, Ito A, Nixon A et al. HDAC6 is a microtubule-associated deacetylase. Nature 2002; **417:** 455–8.
75. Lee YS, Lim KH, Guo X, Kawaguchi Y, Gao Y, Barrientos T et al. The cytoplasmic deacetylase HDAC6 is required for efficient oncogenic tumourigenesis. Cancer Res 2008; **68:** 7561–9.
76. Zhang Z, Yamashita H, Toyama T, Sugiura H, Omoto Y, Ando Y et al. HDAC6 expression is correlated with better survival in breast cancer. Clin Cancer Res 2004; **10:** 6962–8.
77. Saji S, Kawakami M, Hayashi S, Yoshida N, Hirose M, Horiguchi S et al. Significance of HDAC6 regulation via oestrogen signaling for cell motility and prognosis in oestrogen receptor-positive breast cancer. Oncogene 2005; **24:** 4531–9.
78. Hsieh TH, Tsai CF, Hsu CY, Kuo PL, Lee JN, Chai CY et al. Phthalates stimulate the epithelial to mesenchymal transition through an HDAC6-dependent mechanism in human breast epithelial stem cells. Toxicol Sci 2012; **128:** 365–76.
79. Bosch-Presegue L, Vaquero A. The dual role of sirtuins in cancer. Genes Cancer 2011; **2:** 648–62.
80. Ashraf N, Zino S, Macintyre A, Kingsmore D, Payne AP, George WD et al. Altered sirtuin expression is associated with node-positive breast cancer. Br J Cancer 2006; **95:** 1056–61.
81. Kim HS, Patel K, Muldoon-Jacobs K, Bisht KS, Aykin-Burns N, Pennington JD et al. SIRT3 is a mitochondria-localized tumour suppressor required for maintenance of mitochondrial integrity and metabolism during stress. Cancer Cell 2010; **17:** 41–52.
82. Gao L, Cueto MA, Asselbergs F, Atadja P. Cloning and functional char acterization of HDAC11, a novel member of the human histone deactylase family. J Biol Chem 2002; **277:** 25748–55.
83. Liu H, Hu Q, D'Ercole AJ, Ye P. Histone deacetylase 11 regulates oligodendrocyte-specific gene expression and cell development in OL-1 oligodendroglia cells. Glia 2009; **57:** 1–12.
84. Villagra A, Cheng F, Wang HW, Suarez I, Glozak M, Maurin M et al. The histone deacetylase HDAC11 regulates the expression of interleukin 10 and immune tolerance. Nat Immunol 2009; **10:** 92–100.
85. Gore SD, Weng LJ, Zhai S, Figg WD, Donehower RC, Dover GJ et al. Impact of the putative differentiating agent sodium phenylbutyrate on myelodysplastic syndromes and acute myeloid leukemia. Clin Cancer Res 2001; **7:** 2330–9.
86. Munster PN, Troso-Sandoval T, Rosen N, Rifkind R, Marks PA, Richon VM. The histone deacetylase inhibitor suberoylanilidehydroxamic acid induces differentiation of human breast cancer cells. Cancer Res 2001; **61:** 8492–7.
87. Said TK, Moraes RC, Sinha R, Medina D. Mechanisms of suberoylanilidehydroxamic acid inhibition of mammary cell growth. Breast Cancer Res 2001; **3:** 122–33.
88. Cohen LA, Amin S, Marks PA, Rifkind RA, Desai D, Richon VM. Chemoprevention of carcinogen-induced mammary tumourigenesis by the hybrid polar cytodifferentiation agent, suberanilohydroxamic acid (SAHA) Anticancer Res 1999; **19:** 4999–5005.
89. Huang L, Pardee AB. Suberoylanilidehydroxamic acid as a potential therapeutic agent for human breast cancer treatment. Mol Med 2000; **6:** 849–66.
90. Keen JC, Yan L, Mack KM, Pettit C, Smith D, Sharma D et al. A novel histone deacetylase inhibitor, scriptaid, enhances expression of functional ooestrogen receptor alpha (ER) in ER negative human breast cancer cells in combination with 5-aza 2′-deoxycytidine. Breast Cancer Res Treat 2003; **81:** 177–86.
91. Zhou Q, Shaw PG, Davidson NE. Inhibition of histone deacetylase suppresses EGF signaling pathways by destabilizing EGFR mRNA in ER-negative human breast cancer cells. Breast Cancer Res Treat 2009; 117: 443–51.
92. Sharma D, Saxena NK, Davidson NE, Vertino PM. Restoration of tamoxifen sensitivity in oestrogen receptor-negative breast cancer cells: tamoxifen-bound reactivated ER recruits distinctive corepressor complexes. Cancer Res 2006; **66:** 6370–8.
93. Sabnis GJ, Goloubeva O, Chumsri S, Nguyen N, Sukumar S, Brodie AM. Functional activation of the oestrogen receptor-alpha and aromatase by the HDAC inhibitor entinostat sensitizes ER-negative tumours to letrozole. Cancer Res 2011; **71:** 1893–903.
94. Yang X, Phillips DL, Ferguson AT, Nelson WG, Herman JG, Davidson

NE. Synergistic activation of functional oestrogen receptor (ER)-alpha by DNA methyltransferase and histone deacetylase inhibition in human ER-alpha-negative breast cancer cells. Cancer Res 2001; **61:** 7025–9.
95. Fan J, Yin WJ, Lu JS, Wang L, Wu J, Wu FY et al. ER alpha negative breast cancer cells restore response to endocrine therapy by combination treatment with both HDAC inhibitor and DNMT inhibitor. J Cancer Res Clin Oncol 2008; **134:** 883–90.
96. Fackler MJ, Rivers A, Teo WW, Mangat A, Taylor E, Zhang Z et al. Hypermethylated genes as biomarkers of cancer in women with pathologic nipple discharge. Clin Cancer Res 2009; **15:** 3802–11.
97. Veronesi U, Mariani L, Decensi A, Formelli F, Camerini T, Miceli R et al. Fifteen-year results of a randomized phase III trial of fenretinide to prevent second breast cancer. Ann Oncol 2006; **17:** 1065–71.
98. Kim MS, Blake M, Baek JH, Kohlhagen G, Pommier Y, Carrier F. Inhibition of histone deacetylase increases cytotoxicity to anticancer drugs targeting DNA. Cancer Res 2003; **63:** 7291–300.
99. Fuino L, Bali P, Wittmann S, Donapaty S, Guo F, Yamaguchi H et al. Histone deacetylase inhibitor LAQ824 down-regulates Her-2 and sensitizes human breast cancer cells to trastuzumab, taxotere, gemcitabine, and epothilone B. Mol Cancer Ther 2003; **2:** 971–84.
100. Bali P, Pranpat M, Swaby R, Fiskus W, Yamaguchi H, Balasis M et al. Activity of suberoylanilidehydroxamic acid against human breast cancer cells with amplification of her-2. Clin Cancer Res 2005; **11:** 6382–9.
101. Huang X, Wang S, Lee CK, Yang X, Liu B. HDAC inhibitor SNDX-275 enhances efficacy of trastuzumab in erbB2-overexpressing breast cancer cells and exhibits potential to overcome trastuzumab resistance. Cancer Lett 2011; **307:** 72–9.