Clinical and Therapeutic Implications of Histone Acetylation in Breast Cancer
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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 histone 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

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 protooncogenes, 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...
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. 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 bromo domain 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 p21WAF1/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 siruitohs Sir2 (tu), SIRT1–7. Localization of these members either in the nucleus or cytoplasm is still unclear. 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
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 oncoprotein 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>
<thead>
<tr>
<th>HDAC Types</th>
<th>Localization</th>
<th>Functions</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I HDAC 1, 2, 3, 8</td>
<td>Nucleus, Cytosol</td>
<td>Mediate gene repression in response to DNA damage, cell proliferation, cell cycle control and apoptosis</td>
<td>Over-expression</td>
</tr>
<tr>
<td>Class IIA HDAC 4, 5, 7</td>
<td>Tissue specific</td>
<td>Differentiation and development of vascular, cardiac, immune and nervous systems</td>
<td>Over-expression and mutation</td>
</tr>
<tr>
<td>Class IIB HDAC 6, 10</td>
<td>Cytoplasm, Nucleus and cytoplasm</td>
<td>Development of breast stem cells Not known</td>
<td>Over-expression</td>
</tr>
<tr>
<td>Class III (sirtuins) SIRT 1, 2, 3, 4, 5</td>
<td>Nucleus, Cytosol, Mitochondria</td>
<td>DNA repair, oxidative stress, metabolism and ageing</td>
<td>Variable</td>
</tr>
<tr>
<td>Class IV HDAC 11</td>
<td>Nucleus</td>
<td>Development of oligodendrocyte and immune system response</td>
<td>Not known</td>
</tr>
</tbody>
</table>
Histone Acetylation in Breast Cancer Metastasis

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). Histone 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 reprimor, 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 Class I and Class II HDACs (82). HDAC11 expression is high in the brain, kidney, testis, skel-
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

eral tumour types. Hum Mutat 2012; 33: 100–3.
73. Yang X, Phillips DL, Ferguson AT, Nelson WG, Herman JG, Davidson


