# The role of epigenetics in breast cancer progression

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## *Introduction*

Breast cancers are the most common malignancies and the leading cause of cancer death in women worldwide, compromising approximately 22.9% of invasive cancers in women[1]. Incidence rates of breast cancer have continued to increase and several risk factors that are involved in breast cancer have been reported such as geographical variation and diet[2]. There are several types of breast cancer and they fall into different classes including non-invasive, invasive and metastatic breast cancer. Two types of non-invasive breast cancer exist (ductal and lobular, named according to area where breast cancer originates from) and they may progress to invasive breast cancer if untreated.

Breast cancer can be also classified according to presence of estrogen receptor in the mammary gland. Estrogen plays an essential role in mammary epithelial cell division and gland development as well as breast cancer initiation and progression, through its role in gene activation. The biological actions of estrogens are mediated by two members of the nuclear receptor family, estrogen receptors α (ERα) and β (ERβ). ERs bind to estrogen response elements (EREs) facilitating recruitment of various transcription factors and ER coregulators leading to gene activation[3].

Genetic mutations such as amplifications, deletions and point mutations that are linked to development and progression of breast cancer have been extensively studied in the last decades. Patients carrying mutations in the tumor suppressor genes BRCA1 and BRCA2 have a life risk of developing breast cancer as high as 87%[2]. BRCA1 and BRCA2 are associated with maintenance of genomic stability and involved in double-strand DNA repair mechanism[4]. Approximately 5 to 10% of breast cancer cases are considered to be hereditary and about 30–50% of hereditary breast cancers are due to mutations, inherited in an autosomal dominant manner, in BRCA1 and BRCA2. Next to the well-known BRCA mutations, genetic defects in the phosphatidylinositol 3-kinase (PI3K) are involved in aberrant signal transduction pathways in breast cancer. PI3K belong to a family of enzymes that are involved in key cellular functions such as growth, proliferation, survival, angiogenesis, and motility. In several studies, it has been shown that PI3KCA (catalytic subunit of PI3K) and tumor suppressor PTEN (negative

regulator of PI3K) are mutated in several breast tumors and breast cancer cell lines[5,6]. Unlike mutations in BRCA genes, activating mutations in PI3KCA and inactivating mutations in PTEN are present in a broad range of tumor types and might be more general tumorigenic defects. Next to the many genetic alterations that are found over the years, it became apparent that epigenetic changes in the genetic programs have drastic effects on tumor onset and progression.

The term of 'epigenetics' is introduced by C.H. Waddington to in 1942 to name interaction between genes and their products that allow the phenotypic expression. This implies that environmental factors can contribute to the expression of genes[7]. Epigenetics is used to describe collective heritable changes in gene expression that are not due to any alterations in DNA sequence. At first it has been thought that epigenetic information was limited to cell division but it became apparent that epigenetic traits can be transferred to the next generations[8]. Epigenetics was originally described in plants but is found to be a general mechanism in eucaryotic organisms[9–11]. Nowadays epigentics is refered to as the collection of chemical modifications that are present on chromatin that can influence the transcription and/or metabolism of the underlying DNA sequence.

## *Organization of human genome*

The basic units of chromatin are nucleosomes, which consists of 145-147 base pairs of DNA wrapped around an octamer of histone proteins. The histone octamer contains two molecules of each of the core histones: H2A, H2B, H3, and H4. Folding of the DNA in nucleosome structures provides six to seven fold linear compaction and as such allows the cell to pack roughly two meters of DNA in a 6µm nucleus[12].

The highly conserved core histone proteins contain a carboxy-terminal histone fold domain, comprises three α-helices connected by two loops which forms the interior core of nucleosome particle and allows heterodimeric interaction between histones[13,14]. Additionally, each core histones comprises an amino-terminal tail that is highly flexible and protrudes from the nucleosome structure into the environment. These amino terminal tails are subjected to several types of post-translational modifications

(PTMs), including methylation, acetylation, phosphorylation, ubiquitination and ADP-ribosylation[15]. These posttranslational modifications of histones have important roles in nucleosome stability and contribute to the state of the chromatin fiber and higher order structures[16].

The chromatin fiber can be divided into two states; (1) euchromatin, a decondensed form, which provides accessibility of transcriptional machinery to the DNA, and (2) heterochromatin, typically highly condensed and inaccessible, respectively[17]. The nucleosomal structure is important for primary compaction of DNA. Additional compaction of DNA results from interaction of nucleosomes with each other[18]. It has recently demonstrated that nucleosome positioning is determined at least partially by the DNA sequence, indicating that DNA sequence itself also has an important role in determination its accessibility[19].

Chemical modifications on chromatin play an important role in the regulation of hetero-, and euchromatin. These modifications can be achieved by specific enzymes and cofactors and they can be broadly classified into writer and erasers, depending on whether they add or remove an epigenetic mark. Histone acetyltransferases (HATs), histone methyltransferases (HMTs) and DNA methyltransferase (DNMTs) fulfill their function as writers due to their ability adding chemical modifications either on histone or on DNA. In turn, proteins that have opposite affect to writers are histone deacetylaserases (HDACs) and enzymes that remove methyl marks on histones[20].

In order to recognize the chemical modifications, specialized reader proteins that bind to a specific epigenetic mark are present. They are defined by their characteristic reader module; well-studied examples include the methyl-CpG–binding domain for 5-mC–DNA, the bromodomain for lysine acetylation, the chromodomain and tudor, MBT, WD40-repeat and PHD-finger domains, which target methylated lysines or arginines in a residue and methylation statespecific manner[21]. These ‘writing’, ‘reading’ and ‘erasing’ activities, in turn, establish the optimal local environment for chromatin-templated biological processes via the recruitment of large protein complexes to DNA, such as transcriptional regulation and DNAdamage repair[20].

Besides chromatin modifications, the remodeling of nucleosomes is the other dynamic process that can change the chromatin architecture. These so-called remodelers act to increase the DNA accessibility to the regulatory transcription machinery proteins, and thereby control gene expression. Mechanisms of chromatin remodeling include dynamic interplay between ATP-dependent complexes and histone-DNA interactions. Chromatin remodelers cause unwrapping DNA by sliding nucleosomes to different positions (sliding) which changes the accessibility of nucleosomal DNA to transcription factors (TFs)[22].
Chromatin remodelers give complexity to regulation of transcription and the other chromatin template processes[23].

Both genetic and epigenetic events can control the initiation and progression of cancer. Genetic alterations are impossible to reverse however, epigenetic alterations are reversible through the inhibition of either the writer or the erasers of the modification of interest. This reversal of epigenetic aberrations for example allows regression of malignant cell population to a more normal state. Here we review the most current knowledge regarding the role of epigenetic regulators such as chromatin modifications and remodeling complexes in breast cancer progression and their potential for drug targeting.

## *Histone modifications in breast cancer*

### Histone acetylation

Acetylation is the best characterized chromatin modification and involved in transcription, chromatin structure, DNA repair and cancer progression[20]. Transferring acetyl groups from acetyl coenzyme A (acetyl-CoA) to the lysine ε-amino group onto the histones is strongly associated with an open chromatin conformation. Acetylation induces weak electrostatic interaction between histones and DNA by the charge neutralization of the positively charged lysines and consequently increases chromatin accessibility[24]. Consistent with this, it has been revealed that histone acetylation mainly occurs at the promoter and transcribed regions of active genes. In addition, acetylation serves as a platform for recruitment of many bromodomain containing proteins which recognize this modification and modulate the chromatin state[21]. Acetylation is highly dynamic and is governed by the opposing effects of two enzymatic families: the histone acetyltransferases (HATs) and the histone deacetylases

(HDACs). Disruption of the balance between HAT or HDAC activity can lead the development of several types of cancer[25].

## Histone acetyltransferases (HATs) in breast cancer

HATs were the first enzymes shown to be able to modify histone proteins[26]. There are three main HATs families based on their highly conserved structural motifs: the GNAT family (Gcn5-related N-acetyltransferase), the MYST family (MOZ/YBF2/SAS2/TIP60/HBO1), and the p300/CBP family[27,28]. Histone acetyltransferases (HATs) exist as multi-subunit complexes generally consisting of a catalytic subunit and auxiliary proteins such as transcription coactivators and co-repressors. Many HATs have been reported to be associated with breast cancer. Among them, p300/CBP is the most extensively studied and best characterized HATs in breast cancer progression.

***p300/CBP family*** p300 and cyclic AMP response element-binding protein (CBP) are often referred as a single entity although the two proteins share only 63% homology at the amino-acid level. These proteins play important roles in a number of common physiological processes, including proliferation, differentiation and apoptosis[29]. Beside this, mounting evidence revealed that there are a large number of differences between these two proteins in functional manner. p300/CBP were identified by their interaction with the adenoviral E1A oncoprotein[30,31] and more recent findings suggest that they have a strong link to cancer progression through their ability to transcriptionally activate several oncoproteins and tumor-suppressor proteins.

p300/CBP proteins have an intrinsic HAT activity and target more than 70 proteins including themselves[32]. It has been shown that p300/CBP can acetylate K18 and K27 on histone H3[33,34]. This in turn serves to recruit chromatin remodelers and adaptor proteins to establish a scaffold for transcription machinery elements, such as TFIIB and hypophosphorylated RNA polymerase II, as well as for the other HATs such as SRC-1[35,36]. Several *p300/CBP* mutations which are involved in cancer progression are reported[37]. Gayther *et al*[38] showed that truncated mutations in p300 were identified that eliminate HAT activity or interactions with other

proteins such as the p53 tumor suppressor in breast and colon cancers in humans. These truncated mutation leads to inactivation of the second allele and loss of function. This may suggest that p300 normally functions as a tumor suppressor.

p300/CBP play important roles in transcriptionally co-activation of several nuclear proteins involved in cell proliferation, cell cycle regulation, apoptosis, and DNA damage response. These include the oncoproteins such as c-Jun and c-Fos and the tumor suppressor proteins such as E2F, pRb, p53 and BRCA1[37]. p300 also acts as a transcriptional co-activator for HIF-1α (hypoxia inducible factor 1alpha) in breast cancer through its ability to acetylate histones and therefore increase the DNA accessibility. Overexpression of HIF-1α leads increase proliferation[39] and is associated with poor prognosis in breast cancer due to less sensitivity to ionized radiation[40]. p300 is required for full activation HIF-1α. p300 interacts with activation domain of HIF-1α within its C-terminal domain. This interaction increases transactivation of HIF-1α by increasing histone acetylation, resulting in local remodeling of chromatin structure[41]. It has been shown that p300 levels are often up-regulated in invasive breast cancers. The competition between p53 and HIF-1α for binding to p300 determines the activation of HIF-1α downstream genes such as GLUT-1. This may indicate that expression of HIF-1α downstream genes is dependent on both p300 levels and availability in breast cancer[42].

Acetylation of histones by p300/CBP also modulates nuclear factor κB (NF-κB) mediated inflammation. NF-κB is considered an oncogene due to its ability to activate growth promoting and anti-apoptotic genes. Besides, it can also promote metastasis. NF-κB can recruit HATs to the promoter regions of genes upon binding to the DNA, thereby changing the acetylation status of histones at these loci. NF-κB binds to p300/CBP through the transcriptional activation domain of p65 (a subunit of NF-κB). This interaction guides p300/CBP to the gene promoter and is essential for NF-κB mediated gene expression[43]. *IL-8* (interleukin 8), *IL-6* (interleukin 6) and *ELAM* (endothelial leukocyte adhesion molecule) are some examples for NF-κB target genes which are acetylated by p300/CBP[44]. These genes are also reported to be upregulated in breast cancer. IL-8 is considered a potential metastasis factor and its expression is correlated with tumorigenesis in breast cancer[45]. It has been reported that the expression level of IL-8 is negatively correlated with the ER status and is expressed mostly in invasive cancer. Together

this suggests a potential link between invasiveness and IL-8 expression in breast cancer[46]. Beside of this, IL-6 is overexpressed in aggressive breast cancer and regulates several signaling pathways that promotes self- renewal and hypoxia survival in breast cancer[47]. These findings suggest that p300/CBP recruitment to the promoters of specific genes may play important roles NF-κB-mediated inflammation in breast cancer progression.

p300/CBP can also bind to the regions that are occupied by Polycomb-mediated H3K27me3. However, this binding is not able to increase the accessibility of DNA[48]. A possible antagonism between H3K27ac and H3K27me3 may be involved in switching between active and repressed chromatin states and affect the expression level of genes associated with breast cancer, such as tumor suppressors and oncogenes.

Depending on the context, p300 can act either as a tumor suppressor or as an oncogene. This is because its overexpression leads to activate the genes that are important for tumorigenesis, while truncated mutation in p300 eliminate its interaction with tumor suppressor genes such as p53.

### *MYST family*

The HATS belonging to MYST family are highly conserved proteins and have important roles in diverse events involving gene specific transcription, DNA replication and replication. These enzymes fulfill their functions in multi-subunit protein complexes. Their effect on uncontrolled cell growth and maintaining malignancies are extensively studied, however their roles in breast cancer development is less studied[49]. HBO1, a member of MYST family, acetylates preferentially H4K5 and H4K12 and is implicated in the DNA replication through its interaction with replication factors Orc1 and Mcm2[50]. HBO1 is required for licensing and DNA replication. It has been shown that HBO1 associates with replication origins specifically during G1 phase. These findings show a close link between the replication machinery and HATs[51]. *HBO1* gene locates in the 17q21.3 region which is frequently amplified in breast cancers. On top, HBO1 is found to be overexpressed in breast, ovary, stomach/esophagus, and bladder cancers[52]. HAT activity of HBO1 is a key regulator of DNA replication licensing in mammalian cells and its overexpression may imply a link between cell proliferation and breast cancer.

## Histone deacetylases (HDACs) in breast cancer

HDACs function in opposition to HATs by removing acetyl groups from lysine residues within the histone proteins and restore the positive charge on the side chain, resulting in chromatin compaction and transcriptional repression[53,54]. There are 18 HDACs reported, and these can be subdivided into four families depending on their sequence similarity and cofactor dependency. Class I HDACs (HDAC1, 2, 3, and 8, similar to yeast Rpd3); Class II HDACs (4, 5, 6, 7, 9, and 10, similar to yeast Hda1); Class III HDACs (SIRT1–7, related to yeast Sir2); and Class IV HDAC comprises only one enzyme (HDAC11). Class I, II and IV HDACs share structural and sequence homology. Their catalytic activities depend on a zinc ion. Only Class III HDACs requires NAD+ for catalytic activity[55]. HDACs are components of large multiprotein complexes that repress transcription when targeted to a promoter by inducing a more compact local chromatin organization and removal of the binding sites for bromodomain containing proteins. Like HATs, HDACs also play important role in breast cancer development. It has been reported that recruitment of HDACs to the gene promoters affect several mechanisms involved in cell cycle progression and differentiation.

The T-box transcription factor family (TBX) has been shown to be interacting with several HDACs and this interaction is involved in development of breast cancer. TBX2 and TBX3 are members of this family that are reported to be overexpressed in breast cancer cell lines. Overexpression of these two proteins inhibits senescence and related with oncogenesis. Mutations in TBX3 lead to loss of function and result in underdeveloped or absent mammary glands in mice, suggesting that TBX3 is required for normal breast development[56]. TBX3 has been shown to repress the expression of several tumor suppressor genes including p14ARF that is aberrantly expressed and mutated in breast cancers[57]. Strikingly, it has been shown that TBX3 interacts with HDAC 1, 2, 3 and 5 and this interaction enhances the repression of $p14^{ARF}$ in breast cancer. This may suggest that regulation of gene expression by TBX3 is HDAC dependent and that the repression of $p14^{ARF}$ can be reversed by using HDAC inhibitors. Besides TBX3, TBX2 is amplified and upregulated in BRCA1 and BRCA2 mutant breast cancers[58]. TBX2 has role in maintaining proliferation by negatively regulating the expression of $p21^{WAF1}$ cyclin-dependent kinase inhibitor[59]. Furthermore, it has been revealed that TBX2 represses gene

expression by targeting HDAC1 to the promoter region. Displacement of HDAC1 from p21 promoter by using dominant-negative TBX2 results in increased expression of p21 in melanoma cells and therefore induction of replicative senescence occurs. This suggests that the interaction between HDAC1 and TBX2 plays important role in mechanisms involved in proliferation and senescence in melanoma cells[60]. The homology between TBX3 and TBX2 indicates that HDAC-dependent activity of TBX2 in gene regulation may have roles in breast cancer tumorigenesis. However, there is no evidence whether this interaction exists in breast cancer so far.

ARH1 is an imprinted tumor suppressor gene that is expressed in normal breast and ovarian epithelial cells. ARH1 expression is found downregulated in 41% non-invasive and 70% invasive breast carcinomas[61]. DNA hypermethylation of ARH1, methylation of H3K9 and histone H3 deacetylation result in either complete silencing or inactivation of ARH1[62]. ARH1 activity is negatively regulated through binding of E2F transcription factors (TF) to its promoter region. This binding is reduced by the HDAC inhibitor TSA. Furthermore, it has been shown that multiple HDACs can interact with E2Fs and are involved in downregulation of ARH1 gene expression. These data suggest that TFs and their complex with HDACs have a regulatory role in negatively controlling ARH1 expression in breast cancer[63].

HDAC1, HDAC4 and member of class III HDACs (SIRT 3 and 7) are found overexpressed in breast cancers[64]. Among overexpression of HDACs, several HDAC4 mutations are reported in breast cancer cell lines at significant frequency[65]. HDAC6 expression is elevated after estradiol treatment in ER-positive breast cancer MCF-7 cells and can be decreased by using ER antagonist Tamoxifen[66]. Furthermore, patients with high levels of HDAC6 mRNA tended to be more responsive to endocrine treatment than those with low levels, indicates positive association between HDAC6 levels and response to endocrine therapy[67]. HDAC1 interacts with transcription activation function domain 2 (AF-2) of ER-α and suppresses the ER-α activity. This may suggest that HDAC1 induces reduction in transcriptional activity of ER-α, leading to the increased cellular proliferation and tumorigenicity of breast epithelial cells[68].

Taken together, expression level of HDACs and their recruitment to the specific gene promoters by transcription factors play important role in tumor progression and bypass cell

death. This is established mainly through the deregulation of oncogenes and tumor suppressors that are involved in development of breast cancer.

## Histone methylation

Methylation as a histone modification is more complex than any other modification on histones known so far. Methylation can occur on lysine and arginine residues on histone proteins. Lysines can be mono- (me1), di- (me2) or tri- (me3) methylated, whereas arginines can be methylated mono- (me1) and di- (me2), with either both methyl groups on one terminal nitrogen (asymmetric dimethylated arginine) or one on both nitrogens (symmetric dimethylated arginine). To date, several methylated sites on the various histones have been reported of which six of them are well characterized: five on H3 (K4, K9, K27, K36, K79) and one on H4 (K20). Addition of methyl marks on histone proteins can either activate or repress the transcription, depending on the residue and degree of methylation. For instance, some modifications (H3K4, H3K9 and H3K79) are associated with activation of transcription and others with repression. Besides, the degree of methylation is also important, for example H3K9me1 can be found at active genes, whereas H3K9me3 is only associated with gene repression[69].

### Histone methyltransferases in breast cancer

Histone methyltransferases (HMT) are histone-modifying enzymes, (including histone-lysine N-methyltransferase and histone-arginine N-methyltransferase), that catalyze the transfer of the methyl groups to lysine and arginine residues of histone proteins. HMTs exist as multisubunit complexes that contain a catalytic subunit and auxiliary proteins such as transcription factors, co-activators and co-repressors. The SET domain is the catalytic subunit of all histone methyltransferases (HMTs), except for the H3K79-specific DOT1L methylase, and possesses methyltransferase activity. Methylation of histones depends on Sadenosylmethionine (SAM or AdoMet) as the methyl donor. Although there are many factors involved in histone methylation that have been described to be related to cancer in general, only the most relevant ones in the light of breast cancer will be discussed below.

#### *MLL 1-4*

Mixed linkage leukemia methyltrasferases (MLL1-4) are histone H3 at lysine 4-specific methylases that regulate gene activation. Methylation of H3K4 is associated with an active chromatin structure that is permissive to recruit the transcription machinery to gene promoters. They are associated with various oncogenic transformations including myeloid and lymphoid leukemia[70,71]. In humans, there are several MLL families of proteins, such as MLL1, MLL2, MLL3, and MLL4 that fulfill H3K4-specific HMT activity. MLL-associated HMT activity appears to be functional only in the context of their multi-protein complexes with several common protein subunits ASH2L, WDR5, RBBP5 and DPY30[72]. MLLs are important players in cell cycle regulation and stress response. It has been demonstrated that inactivation of MLL1 causes downregulation of cell cycle regulatory genes due to loss of H3K4 methylation at their promoter regions. Consistent with this data, knockdown of MLL1 results in cell cycle arrest at G2/M phase, suggesting its critical role in cell cycle progression[73]. In addition to the cell cycle regulatory role of MLL1, the MLL3 and MLL4 are shown to be co-activators of p53mediated transcription. MLL3/4 methylate H3K4 on the promoter of p53 target genes and induce their endogenous expression in response to the DNA-damage[74]. MLL proteins are found to interact with nuclear receptors (NRs) including ERs and NR co-regulatory complexes, and as such play critical roles in the regulation of hormone-responsive genes[72].

MLLs are reported as master regulators of Homeobox (HOX) genes. There are several *HOX* genes that are reported to be transcriptionally activated by estrogen via involvement of histone methylases MLL1-4. *HOX* genes play critical roles in cell differentiation and embryonic development. Besides, HOX proteins are also associated with oncogenic transformation of hematopoietic cells. Amongst them, HOXC6 is a critical player in mammary gland development and milk production, and importantly is overexpressed in breast cancers[75]. It has been shown that there are two putative EREs in *HOXC6* promoter and knockdown of ER abolishes the recruitment of MLL2 and MLL3 to *HOXC6* promoter suggesting that MLL2 and MLL3 are recruited to the *HOXC6* promoter in an ER-dependent manner[76]. The HOX13 promoter also contains several EREs which are close to transcription start site and knockdown of ER affects the estrogen dependent binding of MLL indicatingthat estrogen play critical role in recruitment of MLL to the promoter of *HOX13*[77]. Taken together, these results suggest that the MLL complexes play critical roles in transcriptional activation of *HOX* genes by estrogen and that the MLL proteins may be interesting targets in estrogen responsive breast tumors.

### *SMYD3*

SMYD3 is a methyltransferase which is specifically responsible for di- or trimethlylation of H3K4[78]. SMYD3 encoded a 488 amino acid protein containing a SET domain which serves catalytic activity, a zf-MYND domain and a SET-N region. Mutations in the conserved sequence of the SET domain result in abolished methyltransferase activity. Methylation of H3K4 by SMDY3 results in an open chromatin confirmation and provides a platform for the transcription machinery. It has been shown that SMYD3 interacts with HSP90A (heat shock 90kDaprotein 1α) and this interaction is important for maintenance of methyltransferase activity of SMYD3[79].

*SMYD3* expression is elevated in great majority of breast tissues and knockdown of SMYD3 by siRNA results in growth inhibition of breast cancer cell lines, suggesting that high SMYD3 expression is essential for proliferation of breast cancer. In same study, it has been shown that SYMD3 positively regulate the expression of proto-oncogene WNT10B[80]. Aberrant activation of the canonical Wnt/β-catenin pathway is one of the most frequent signaling abnormalities in human cancer[81]. Wnt signals are strongly implicated in initial development of the mammary rudiments, ductal branching and alveolar morphogenesis that occurs during pregnancy[82]. These results indicate that regulation of WNT10B by SMYD3 may drive the proliferation of breast cancer cells.

SMYD3 levels are transcriptionally enhanced by E2F1. Three tandem repeats of E2F-1– binding element is located in the SMYD3 promoter region. The high number of tandem repeats of the E2F-1–binding site in the promoter regions of SMYD3 leads its overexpression and is associated with high risk of developing human colorectal, hepatocellular and breast cancer[83]. E2F1 is a member of the family of E2F transcription factors. E2F is crucial in cellcycle control and DNA synthesis through transcriptional activation of a number of downstream genes[84]. Taken together, suppression of the activity of SMYD3 could reduce the risk of developing several tumors, including in breast tissue.

### *EZH2 (KMT6)*

EZH2 is a catalytic component of polycomb repressive complex 2 (PRC2) and an orthologous of the Drosophila chromatin repressor protein 'enhancer of zeste'. EZH2 is a highly conserved histone methyltransferase that is responsible for the methylation of lysine 27 of histone H3 (H3K27) and, to lesser extent H3K9 both in vivo and in vitro[85]. These histone marks are usually associated with a transcriptionally silent state[86]. EZH2, the catalytic subunit of the PRC2 complex, catalyzes trimetylation of HK27 around polycomb response element (PRE). Addition of methyl mark leads to recruitment of polycomb repressive complex 1 (PRC1) through its chromodomain. PRC1 exerts E3 ubiquitin ligase activity through its RING1b domain. Once PRC1 is recruited to H3K27me3, RING1b adds ubiquitin to H2AK199 which results in a compact chromatin state[87]. These combined activities of PRC2 and PRC1 are important for induction/maintenance of transcription repression.

EZH2 levels are significantly increased in invasive breast carcinomas and breast cancer metastases at both the transcript and protein levels when compared with normal breast tissues[88]. It has been found that polycomb proteins are involved in silencing of tumor suppressor protein $p16^{INK4A}$ by using human mammary epithelial cell (HMEC) system which allows to investigate transition from a premalignant to malignant state[89]. Consistent with this study, Hinshelwood *et al*.[90] found that tumor suppressor $p16^{INK4A}$ is enriched in trimethylated H3K27 in pre-selection HMECs. Both EZH2 and BMI-1 (a member of PRC1) are overexpressed in post-selection HMECs and in breast tumor cells[91] clearly showing that overexpression of polycomb proteins silences tumor suppressors and this results indicate their importance in development of breast carcinomas.

### *PRDMs*

PRMDs (PR domain containing genes, with zinc fingers) are defined by the presence of PR domain which is nearly 30% identical to the SET domain found in many histone lysine methyltransferases. They play important roles in development, differentiation and disease such as cancer. Several PRMD members are reported to be involved in breast cancer, including PRMD2 and 14.

PRMD2 catalyzes methylation of H3K9 but on the other hand can acts as a co-activator of the estrogen receptor by recruiting HATs to the promoter of estrogen receptor target genes, resulting in transcriptional activation. In the absence of estrogen, PRMD2 locates on estrogen target genes through direct protein-DNA interaction and represses transcription through methylation of H3K9. In the presence of estrogen, ER binds to PRDM2 and switches it from direct binding to DNA into indirect binding as mediated by ER. The ER-bound PRDM has lower H3K9 methylation activity and cooperates with other co-activators to stimulate transcription[92], suggesting PRDM2 may have important role in ER-positive breast cancer however the genes which are regulated by PRDM2 in an estrogen dependent manner are unknown.

PRDM14 is also enhanced cell growth and consistently its knockdown by siRNA increases sensitivity to chemotherapeutic drug, thereby inducing apoptosis in breast cancer cell lines[93]. PRDM14 was identified as an important actor in self-renewal and differentiation of embryonic stem cells (ES)[94]. This data suggests that overexpression of PRDM14 might have important roles in proliferation of breast cancer cells. In the same study, it was found that PRDM14 induced growth by activated H-ras. Together suggesting that PRDM14 may act in concert with a growth signal to facilitate cellular transformation in breast cancer cell lines[93].

## Histone demethylation in breast cancer

### *PLU-1 (JARID1B)*

PLU-1 is a nuclear protein that shows a highly restricted expression profile in normal adult tissues but which is aberrantly expressed in breast cancer cell lines and tissues[95]. PLU-1 is a

Jumonji C domain containing protein and fulfills the catalytic activity to remove methyl groups specifically from H3K4. All methylation states (trimethyl, dimethyl, monomethyl) of H3K4 can be targeted by PLU-1 in MCF-7 cells and reduction in the level of H3K4me at the gene promoters is associated with transcriptional repression of certain genes. Yamane et. al.[96] showed that knockdown of PLU-1 reduces the growth rate due to a defect in G1/S transition and results in upregulation of breast cancer related genes including BRCA1, CAV1, 14-3-3 sigma and HOXA5 in MCF7 cells, consistent with the data shows PLU-1 can act as a

transcriptional repressor[97]. More recently, PLU-1 is shown to have an essential role in early stage of embryonic development in mouse. Deletion of the ARID domain (AT rich DNA binding domain) of PLU-1 results in a reduction of terminal end bud development and side branching phenotype in mammary gland. This phenotype is also observed in estrogen receptor α (ERα) knockout mouse[98]. Furthermore, it has been shown that PLU-1 interacts with ERα and that knockdown of PLU-1 leads to reduction of tumor size in mammary gland, indicating that PLU-1 is involved in ERα stimulated growth of ER-positive breast cancer cells[99]. Taken together, demethylation activity of PLU-1 and its interaction with ERα may contribute to change the expression levels of the genes that are involved in cell growth. PLU1 acts as a tumor suppressor and plays important roles in cell proliferation in mammary gland.

### *GASC1 (JMJD2C)*

Gene amplified in squamous cell carcinoma 1 (*GASC1)* is amplified and overexpressed in multiple breast cancer cell lines[100]. It has been shown that GASC1 has a Jumonji C domain that catalyzes demethylation of specifically H3K9 and H3K36. Demethylation of H3K9 is associated with active gene expression[101,102]. GASC1 is also associated with maintaining gene expression programs that are important for self-renewal and differentiation in ES cells[103]. Additionally, overexpression of GASC1 results in phenotypic alterations such as growth factor-independent proliferation and anchorage-independent growth in human nontransformed mammary epithelial cells, suggesting a potential role in breast cancer progression. The *NOTCH1* has been shown as a target gene of GASC1[100]. NOTCH1 signaling pathway has been implicated in a number of cellular properties important in cancer, including cell division and survival. In addition, it is essential for self-renewal of stem cells, including stem, and/or progenitor cells isolated from the mammary gland where *GASC1* is shown to be overexpressed[104,105]. These data may suggest that histone demethylase activity of GASC1 leads to overexpression of *NOTCH1* and this may be associated with a stem cell phenotype in breast cancer.

### *JMJD2B*

Jumonji domain containing 2B (JMJD2B), a member of histone demethylase JMJD2 family, specifically target H3K9me3 for demethylation at pericentric heterochromatin and euchromatin. Yang *et al*.[106] showed that both mRNA and protein levels of JMJD2B are found

to be higher in ER-positive (T47D and MCF7) cell lines in comparison with ER-negative (MDAMB-231 and MDA-MB-468) cell lines. Treatment with an ERα inhibitor reduces the expression level of JMJD2B, supporting JMJD2B may be an ER-dependent gene[107]. Furthermore, it has been revealed that Jumonji-C domain of JMJD2B interacts with ERα. This interaction increases after estrogen treatment. Next to being a target for ER, JMJD2B is recruited to the ER binding site which leads to a reduction in H3K9me3 levels, suggesting JMJD2B mediates the induction of ER target genes and estrogen-dependent proliferation of breast cancer cells. JMJD2B mediates the expression of ER target genes including *MYB* and *GREB1* through its demethylase activity. High induction of these genes is associated with cell proliferation and survival which may have roles in tumorogenesis in breast cancer. Consistent with this, inactivation of JMJD2B leads to decrease tumor growth rate in ERpositive breast cancer[108]. These data indicate the existence of synergic activity between JMJD2B and ER in regulation of gene expression.

JMJD2B regulates important signaling pathways including Wnt, transforming growth factor-β (TGF-β), vascular endothelial growth factor, angiogenesis and cell cycle, all of which play important roles in breast cancer progression. JMJ2B epigenetically regulates expression level of cell cycle genes such as *CCNA1* (encodes Cyclin A1), *CCND1* (encodes Cyclin D1) and *WEE1*, indicating JMJD2B has important roles in controlling G1 phase progression and G2/M transition in breast cancer cell lines. H3K9me3 levels were significantly enriched on promoter regions of *CCNA1, PGR*, and *PMP22* genes which are related to cell cycle progression when JMJD2B is depleted by siRNA. Loss of JMJD2B causes down-regulation of gene expression and a significant G2-M phase arrest in MCF-7 breast cancer cell line[106].

### *Protein arginine N-methyltransferases (PRMTs)*

PRMTs catalyze the transfer of a methyl group to the side chain nitrogens of arginine residues within proteins[109]. Protein arginine methylation has been implicated in several mechanisms including signal transduction and transcription[110,111]. CARM1/PRMT4 is a histone arginine methyltransferase that specifically methylates H3R17 and H3R27. This ability of CARM1 leads

to a transcriptionally active chromatin state[112]. CARM1 is described as a transcriptional co-activator of NR-associated factors and it is thought that recruitment of CARM1 with these factors can serve as a scaffold to target CARM1 to specific regulated sites where CARM1 can methylate histones and participate in gene activation[113,114]. It has been shown that CARM1 a positive regulator of ERα mediated transcriptional activation. ERα stimulation induces the formation of a complex including CARM1, ERα, and co-activator protein AIB1 on the ERE of the *TFF1* gene where CARM1 can methylate specific arginine residues on histone H3[115]. TFF1 revealed to be important for stimulation of breast cancer cell migration and invasion of surrounding tissues[116]. These findings suggest that arginine methylation by CARM1 leads to activation of *TFF1* and this may have a critical role in determining the pattern of metastatic spread of breast cancer. CARM1 has also been shown to be required for ERα-induced proliferation of breast cancer cells. Upon estrogen signaling, CARM1 and co-activator AIB1 are recruited *E2F-1* promoter and induce dimethylation of H3R17. Activation of *E2F-1* by CARM1 positively regulates induction of E2F1 target genes (*CDC25A, CCNA1, CCNE1,* and *CCNE2*) that are important for cell cycle entry and results in breast cancer cell proliferation[117]. Taken together, arginine methyltransferase CARM1 functions in breast cancer cell proliferation and migration through specific gene activation.

## Other histone modifications in breast cancer

Beside methylation and acetylation, other modifications of histone proteins including phosphorylation, ubiquitination/sumoylation, ADP-ribosylation, deamination, and proline isomerization play important roles in in breast and other cancer types. Although their impact on the gene regulation is less well studied in comparison to histone methylation and acetylation, some studies have shown their mechanism and functions in regulation of genome.

Histone phosphorylation is associated with a variety of cellular processes, including transcriptional regulation, apoptosis, cell cycle progression, DNA repair, chromosome condensation, and regulation of developmental genes[118]. Phosphorylation of serine and threonine residues within the histone tails is linked to chromatin condensation during mitosis and meiosis[119]. There is very little known about the role of histone phosphorylation in cancer development, however mounting evidences suggest that it is involved in DNAdamage repair,

chromosomal stability and apoptosis[20]. Besides these functions of histone phosphorylation, it is also important for transcriptional activation of genes during cell cycle. It has been found that at least 2 serine residues (S10 and S28) of histone H3 are phosphorylated. The effect of these two phosphorylation sites is independent from each other and it promotes gene expression separately[120]. It has been shown that phosphorylation of histone H3S10 residue is increased upon TPA (activator of Ras/MAPK pathway) and these histone H3pS10 sites co-localize with the transcriptionally active chromatin regions in MCF-7 breast cancer cell line. Thus, stimulation of Ras/MAPK by either growth factors or TAP and elevated histone H3 pS10 at transcriptionally active loci may be associated with neoplastic transformation and breast cancer progression[121]. Besides this, histone H3S10 phosphorylation has also been shown to play a role in transcriptional activation of NFkB pathway genes and "immediate early" genes like *c-jun* and *c-fos*[28]. It has been proposed that histone phosphorylation controls the binding of proteins to chromatin[122]. Heterochromatin protein 1 (HP1) is associated with constitutive heterochromatin and transcriptionally inactivation of genes. HP1 binds to H3K9 when it has been trimethylated by HMTs[123]. Aurora B catalyzes the phosphorylation of H3S10 late G2 phase and mitosis and diminishes the binding of HP1 to the adjacent

H3K9me3.Phosphorylation of H3S10 is associated with genes that are transcriptionally active during mitosis. This mechanism is called a 'phospho/methyl switch' and is used to temporary block the binding of reader proteins during cell cycle and as such affect the transcription of the target genes.

Besides of phosphorylation, mounting evidences revealed that ubiquitination of histones plays important roles in carcinogenesis[124]. PRC1 possesses E3 ubiquitin ligase activity that targets ubiquitination of H2A on lysine 119 (H2AK199ubi) which leads to PcG-mediated gene silencing[125]. Besides this, ubiquitination of H2A has been shown to be associated with DNA repair mechanisms. The BMI1 andRING1b, subunits of PRC1, are recruited to sites of DNA double-strand breaks (DSBs) where they contribute to the ubiquitination of γ-H2AX. It has been shown that recruitment of several mediator/repair proteins such as 53BP1 and BRCA1 is abolished and cells are sensitized to ionizing radiation in the absence of BMI1[126]. These results suggest that ubiquitination of H2A by BMI1has an important function in maintaining genomic stability and prevention of tumorigenesis.

E3 ubiquitin ligase complexes can act either as a tumor suppressor or as an oncogene depending on its target proteins in breast tumors (Otha 2004). Recently, it has been shown that inhibition of proteasome complex by proteasome inhibitor bortezomib results in a reduction of H2B monoubiquitination (H2Bub1), leading to transcriptional elongation defects on estrogen target genes and to decreased chromatin dynamics overall in MCF-7. Moreover, a dramatic loss of H2Bub1 during tumor progression may lead to estrogen-independent cell proliferation and increased metastatic properties[127].

## *DNA methylation*

DNA methylation is an extensively studied epigenetic modification in which a methyl group is put on the 5-carbon of cytosine (5mC) in CpG dinucleotides. In the context of DNA methylation, sequences within the genome can be divided into two different classes: CpG islands and CpG poor regions. Definition of CpG island is a region of at least 500 bp, and having GC content greater than 55%[128]. CpG islands are frequently found at the 5' ends of the regulatory regions of many genes (promoter, untranslated region and exon 1). The majority of the genome, such as the intergenic and the intronic regions, is known to be CpG poor[129].

DNA methylation is catalyzed by a group of enzymes known as the DNA methyltransferases (DNMT). DNMT1 is the best known and studied member of the DNMT family which is responsible for maintenance of methyl groups that are already present on one of the DNA strands. DNMT1 is localized to the replication fork during cellular division and reproduces DNA methylation patterns from hemi-methylated DNA[130]. DNA methylation is a relatively stable modification that is inherited throughout cellular divisions. The other major class of DNA methyltransferases consists of DNMT3, DNMT3a and DNMT3b, which are essential for de novo methylation of CpG islands in early embryos, during development and carcinogenesis[131]. Several mouse experiments showed that DNMT deficient mice die early in development or immediately after birth underscoring the importance of these enzymes[132]. DNA methylation is associated with long-term silencing[133] and regulates mechanisms which are involved in tissue-specific gene expression in normal cells. DNA methylation can directly influence gene transcription by affecting the binding of transcriptional factors and cofactors to their target

sites or chromatin structure by effecting nucleosome occupancy within the promoter regions of genes[132].

DNMTs are demonstrated to be overexpressed in breast carcinomas. Especially, the *DNTM3B* gene showed the highest expression level in comparison to the *DNMT1* and *DNMT3A*. High expression of DNMT3B is associated with aggressive breast cancer and this indicates its role in cell proliferation[134]. There are several genes that are reported to be silenced by DNA methylation in breast cancer and thus DNA methylation affects several important pathways involved in maintenance of homeostasis in breast tissue, including cell cycle regulation, tumor susceptibility, carcinogen detoxification and cell adhesion[135]. Especially, inactivation of tumor suppressor and cell cycle related genes by promoter hypermethylation has been considered as a potentially important mechanism involved in the development of breast cancer.

The *14–3–3σ* gene is member of a gene family responsible for inhibiting the activities of specific cyclin-dependent kinases and as a consequence of this, causes cells to arrest in G2 cell cycle checkpoint in response to DNA damage[136]. It has been shown that hypermethylation of the *14–3–3σ* gene occurs in a CpG-rich region that extends from the transcriptional initiation site to the middle of the coding region. Methylation-mediated chromatin condensation is responsible for suppressing *σ* transcription instead of genetic alterations such as loss of heterozygosity (LOH) and intragenic mutations in breast cancer[137]. This indicates the impact of methylation of promoter of *14–3–3σ* to breast cancer progression. Overexpression of 14–3–3*σ* blocks cell proliferation and cell cycle entry due to its inhibitory effect on cyclin-CDK activities in many breast cancer cell lines[138]. 14–3–3*σ* has been demonstrated to be downregulated in broad range of cancers such as breast and gastric cancer, suggesting 14–3–3*σ* acts as a tumor suppressor during tumorigenesis.

The CDH1 gene encodes a transmembrane glycoprotein E-cadherin that is important in maintaining cell-cell adhesion in epithelial tissues. The chromosomal region where CDH1 gene is located is often associated with loss of heterozygosity (LOH) in breast cancer[139]. It has been shown that hypermethylation of *CDH1* CpG island occurs in high frequencies in noninvasive breast cancer and expression of E-cadherin protein is significantly reduced in breast cancer[140]. Loss of function of this gene is implicated in reduced tissue integrity and increased metastasis.

Based on these data, gene silencing by DNA methylation results in inactivation of several important genes involved in cell cycle progression and cell-cell adhesion, thereby inducing tumor formation.

## *Chromatin remodelers*

Chromatin remodelers hydrolase ATP for energy production to change the condensed state of chromatin by removing, ejecting or restructuring the nucleosomes. This mechanism increases the accessibility of DNA sequence to regulatory proteins that can allow execution of various processes, including gene transcription, DNA replication, DNA repair, and DNA recombination[141]. There are four major families of chromatin remodeling complexes: the SWI/SNF (switching defective/sucrose non-fermenting) family, the ISWI (imitation SWI) family, the NuRD (nucleosome remodeling and deacetylation)/Mi-2/CHD (chromodomain, helicase, DNA binding) family and the INO80 (inositol requiring 80) family. Although all remodelers share a conserved ATPase domain, each complex has a unique subunit composition[23,142]. Several subunits within the chromatin remodeler complexes have been found to be mutated in malignancies, supporting that they might have tumor suppressor function. Especially, mutations and/or loss of expression of the genes encoding SWI/SNF complex subunits have now been identified in various cancers[143].

*PBRM1* encodes the BAF180 protein, the chromatin targeting subunit of the PBAF SWI/SNF chromatin remodeling complex and maps to the 3p21 region[143] where allele loss is frequently detected in breast cancer[144]. BAF180 plays important role in the regulation of G1/S transition of cell cycle when re-expressed in BAF180 deficient cells. It has been shown that BAF180 binds to promoter region of *p21* and consequently upregulates p21 in breast cancer cell lines[145]. p21 is a potent cyclin-dependent kinase inhibitor and function as a regulator of cell cycle progression at G1. This explains the role of BAF180 in controlling the cell cycle in breast cancer.

BRG1, the catalytic subunit of SWI/SNF complex, is silenced or mutated in several tumors cell lines and a BRG1-associated complex was recently found to co-purify with BRCA1, involved in breast and ovarian cancers[146,147]. It has been shown that BRCA1 can directly interact with the BRG1 subunit of the SWI/SNF complex and p53-mediated stimulation of transcription by

BRCA1 was completely abrogated by a dominant-negative mutant of BRG1[147]. These results may suggest that association of chromatin remodeling complex with a tumor suppressor gene may have role to maintaining genome integrity in preventing breast cancer progression.

ARID1A encoding a human homolog of yeast SWI1 which contains DNA binding domain. It is an internal member of SWF/SNF complex and thought as a tumor suppressor gene since it is found mutated in up to 50% of ovarian clear cell carcinomas[148]. It has been found that low expression of ARID1A is associated with more aggressive breast cancer phenotypes and reexpression of ARID1A in the T47D breast cancer cell line results in significant inhibition of colony formation in soft agar[149]. Furthermore, liganded ER undergoes conformational changes and targets SWI/SNF to the ER response element[150]. Taken together, these evidences suggest that chromatin remodeling complexes are critical for tumorigenesis and transcription in tumors.

## *Modification of non-histone proteins*

Apart from various modifications on histones, many post translational modifications by HATs, HMTs and HDACs have been demonstrated to play critical roles in regulation of nonhistone proteins such as transcriptional factors, cytoskeleton components and many other cellular proteins[151]. Although it is not fully anticipated yet, this mechanism could be an important regulatory step in the control of diverse biological processes on top of the regulation via histones.

p53 was the first acetylated non-histone protein that has been reported. p53 is a tumor suppressor which has crucial roles in cell cycle regulation and prevention tumor formation. Addition of acetyl groups by p300/CBP to the certain lysine residues (largely at K320, K370, K372, K373, K381, K382) within the C-terminus of p53 increases during DNA damage. This induces the activity of p53 as transcription factor and, consequently, increases activation of its target genes[152]. Acetylation of p53 is important also for its stabilization because the lysine residues acetylated in p53 overlap with those that are can be ubiquitinated. MDM2, negative regulator of the p53, can promote p53 deacetylation by recruiting HDAC1-containing complexes and mediate the ubiquitation of deacetylated lysine residues. These functions of

MDM2 ultimately lead to degradation of p53[153]. Recently, it has reported p53 is methylated at the same residues (K370, K372, and K382) that are subjected to acetylation, ubiquitination and sumoylation. Methylation of p53 associated with activation and repression depending upon the status and the site of methylation. K372me1 (by Set9) activates p53 activity and in contrast, K370me1 (by Smyd2) and K382me1 (by SET8) repress p53 activity. Following DNA damage, the K382me1 levels decrease as p53 becomes activated[154–156], therefore the regulation of modifications of p53 is a key in its tumor suppressor function.

The E2F family transcriptional factors is another important group of non-histone protein that is regulated by PTMs. E2F-11 is activated immediately after released from the Rb-E2F-1 repressor complex during G1/S transition and targets several genes that are related in cell cycle control and programmed cell death[84,157]. It has been shown that proliferation promoting members of E2F family (E2F-1, 2, 3) are acetylated by CBP/p300 within the specific lysine residues (K117, K120 and K125). Addition of acetyl groups to the N terminal DNA binding domain of E2F-1 increases its DNA binding affinity and prevents its ubiquitation, consequently leading to the stabilization of the protein[158,159]. E2F-1 mediated apoptosis can be enhanced by acetylation dependent stabilization in response to genotoxic stress[160]. Next to histone modifications, modification of non-histone proteins plays important roles in stabilization of proteins and maintenance of their activities.

## *Epigenetic therapy for breast cancer*

In recent decades, the use of adjuvant therapies for breast cancer has increased extensively and has contributed to the reduction in the mortality of breast cancer[161]. Multidisciplinary therapies are put into practice to treat breast cancer due to diversity of the disease. There are several treatment options including surgery, radiation therapy, cytotoxic chemotherapy, molecularly targeted endocrine therapy and their combinations depending on the type of breast cancer[162]. Besides of these therapies, targeting the specific enzymes that are related to epigenetic mechanisms is emerging as an effective and valuable approach to treat several types of cancer including breast[163] DNMTs and HDACs have become primary target for epigenetic therapy. There are four epigenetic drugs that are approved by Food and Drug Administration (FDA) to date: the DNA methyltransferase inhibitors 5-azacytidine (Vidaza) and

decitabine (20-deoxy-5-aza- cytidine, Dacogen) and the HDAC inhibitors suberoylanilide hydroxamic acid (SAHA, vorinostat,Zolinza) and romidepsin (Istodax)[164].

Inhibition of DNMTs reactivates the genes that have been silenced by DNA methylation and reconstitutes non-carcinogenic status of cells. 5'-aza-2'-deoxycytidine (5-azaCdR) is a cytosine analogue and incorporated into the DNA during the replication. Both DNMT1 and DNMT3B can be inhibited by 5-azaCdR and this enhances the reactivation of tumor suppressor genes. 5-aza-CdR induces tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and increases chemosensitivity to anticancer agents in breast cancer cells[165,166].

Apart from hypermethylation of specific cancer-associated gene promoter sites, overall DNA hypomethylation is also one of the characteristics of human cancer cell[167]. Genomic hypomethylation has been associated with aberrant gene expression of oncogenes, genomic instability and activation of retrotransposons[168]. The effect of DNMTs is not specific for tumor suppressor genes but rather than cause reduced levels of genome-wide DNA methylation. Their ability to induce global DNA hypomethylation could promote oncogenic transformation in cancer patients. It has been shown that DNA demethylation is also important for cellular differentiation and a further decrease in DNA methylation might induce phenotypic variability[169]. It is clear that the identification and development of more specific drugs is critical for epigenetic therapies and this might be achievable through the targeting of other epigenetic factors rather than the inhibition of DNA methyltransferases.

HDAC inhibitors (HDACi) inhibit deacetylation of histones and result in the accumulation of acetyl marks on the histones. It has been shown that HDAC inhibitors have anti-proliferative affect and inhibit cell growth in breast cancer cell lines. SAHA is a small molecule that inhibits HDAC activity. It has received approval for treatment of patients with cutaneous T-cell lymphoma[170]. It has been demonstrated that inhibition of HDACs by using SAHA results in a reduction of proliferation and induces differentiation in breast cancer cell lines[171]. SAHA is currently under evaluation in several phase II trials in breast cancer. It has been shown that using SAHA in combination with other agents such as endocrine therapies or cytotoxic agents significantly improved the therapy response in comparison to treatment with the agent alone[172,173]. Trichostatin A (TSA) is a hydroxamic acid HDAC inhibitor and inhibits growth of

ERα-positive breast tumor and breast cancer cells. TSA synergizes with the demethylating agent EGCG reactivation ERα expression in ERα-negative breast cancer cells[174].

Epigenetic drugs have several advantages such as being effective at low doses and less toxic, however, aberrant patterns can return with the removal of the drug. Additionally, global demethylation and inhibition of histone deacetylation are not selective for cancer-specific epigenetic marks. It has been shown the enzymatic activity of HDACs is not restricted to histone proteins. For instance, inhibition of HDACs by using highly specific small molecules enhances hyperacetylation of 1750 proteins in human cancer cell lines, suggesting that nonhistone proteins constitute the large majority of HDAC substrates[175]. Epigenetic cancer therapy requires further development of enzyme-specific inhibitors to provide sufficient tumor selectivity for therapeutic success.

## *Conclusions*

Systemic treatment of breast cancer is based on endocrine therapy, cytotoxic chemotherapy, and molecular targeted therapy. Since breast cancer is a heterogeneous disease, using adjuvant therapies is providing insight into disease free survival. ER status is very important due to its association with several molecular pathways involved in breast cancer progression. Therapies directed against hormone receptors by antibody based approaches lead to significant improvements to cure breast cancer. However these therapies only take into account breast cancer patients with overexpressed hormone receptor status. Besides, addition of chemotherapy to adjuvant targeted therapy increases the effect of treatment. Identification of new prognostic and predictive markers depending on molecular background of the patient allows curing breast cancer in the most effective way by correctly targeting patients that are most likely to respond to a specific therapy.

It has been proposed that epigenetic is a crucial mechanisms that is associated with cancer progression. Although the exact number and variety of proteins that are related in epigenetic regulation of breast cancer is still unknown, it is clear that far more proteins are involved in this mechanism than initially appreciated. It will be important to elucidate the exact role of key players that are involved in the generation of epigenetic mechanisms related to breast

cancer. Combinational data from cancer genome sequencing project with epigenome sequencing project may allow investigating many additional epigenetic target candidates. It is now becoming increasingly clear that understanding epigenetic mechanisms allow to investigate the mechanisms underlying the phenotypic plasticity of cancer cells and to develop of new therapeutic strategies.

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