



Epigenetic and breast cancer therapy: Promising diagnostic and therapeutic applications

Gulab Sher^{a,1}, Nadia Aziz Salman^{b,1}, Abdul Q. Khan^a, Kirti S. Prabhu^a, Afsheen Raza^c,
Michal Kulinski^a, Said Dermime^c, Mohammad Haris^{d,e}, Kulsoom Junejo^f, Shahab Uddin^{a,g,*}

^a Translational Research Institute, Academic Health System, Hamad Medical Corporation, Doha, P.O. Box 3050, Qatar

^b Kingston University London, School of Life Science, Pharmacy and Chemistry, SEC Faculty, Kingston, upon Thames, London, KT1 2EE, UK

^c National Center for Cancer Care and Research, Hamad Medical Corporation, Doha, P.O. Box 3050, Qatar

^d Functional and Molecular Imaging Laboratory, Sidra Medicine, P.O. Box 26999, Qatar

^e Laboratory Animal Research Center, Qatar University, Doha, P.O. Box 2713, Qatar

^f General Surgery Department, Hamad General Hospital, Hamad Medical Corporation, Doha, P.O. Box 3050, Qatar

^g Dermatology Institute, Academic Health System, Hamad Medical Corporation, Doha, P.O. Box 3050, Qatar

ARTICLE INFO

Keywords:

Epigenetics
Breast cancer
DNA methylation
Epigenetic drugs
Biomarkers

ABSTRACT

The global burden of breast cancer (BC) is increasing significantly. This trend is caused by several factors such as late diagnosis, limited treatment options for certain BC subtypes, drug resistance which all lead to poor clinical outcomes. Recent research has reported the role of epigenetic alterations in the mechanism of BC pathogenesis and its hallmarks include drug resistance and stemness features. The understanding of these modifications and their significance in the management of BC carcinogenesis is challenging and requires further attention. Nevertheless, it promises to provide novel insight needed for utilizing these alterations as potential diagnostic, prognostic markers, predict treatment efficacy, as well as therapeutic agents. This highlights the importance of continuing research development to further advance the existing knowledge on epigenetics and BC carcinogenesis to overcome the current challenges. Hence, this review aims to shed light and discuss the current state of epigenetics research in the diagnosis and management of BC.

1. Introduction

Cancer is a significant global health concern. In 2018, an estimate of 18.1 million new individuals were diagnosed with cancer alongside 9.6 million mortalities [1]. By 2040, these numbers are expected to double, particularly in low and middle-income countries. Consequently, the burden of cancer on healthcare systems is likely to immensely increase worldwide [2]. This highlights the need for more research to further advance an early and rapid detection and management of this disease which serve as a key role of improving survival rates and patient-centered cancer care [3].

Breast Cancer (BC) is one of the most common diagnosed female

cancers and leading cause of cancer death among women, accounting for an estimate of 627,000 (6.6 %) deaths worldwide [4]. Since 2008, BC incidence and mortality rates have increased globally by more than 20 % and 14 % respectively. The global BC burden is estimated to have risen to 2.1 million new cases in 2018 compared to nearly 1.7 million in 2012 [1,5].

The high incidence and death rates in BC are linked to various factors, among which the most common being its heterogeneous nature. The inter/intra-tumoral heterogeneity, usually affecting one anatomic site of the breast with phenotypic and molecular diversity, plays a key role in its histology and staging [6]. The molecular stratification of BC is primarily based on gene expression profiling; this also includes the

Abbreviations: MeCP2, Methyl-CpG-binding protein 2; H3, Histone 3; H4, Histone 4; TNBC, Triple negative breast cancer; ER α , Estrogen receptor α ; PTEN, Phosphatase and tensin homolog; ER β , Estrogen receptor beta; CAF, Cancer-associated fibroblasts; MMPs, Matrix metalloproteinases; TGF- β , Transforming growth factor- β ; KMT2D, Lysine methyltransferase 2D; HDAC, Histone deacetylases; KDM7A, Lysine demethylase 7A; BCSCs, Breast cancer stem cells; ctDNA, Cell-free tumor DNA; LN, Lymph node.

* Corresponding author at: Translational Research Institute, Dermatology Institute, Academic Health System, Hamad Medical Corporation, P.O. Box 3050, Doha, Qatar.

E-mail address: skhan34@hamad.qa (S. Uddin).

¹ Joint First Author.

<https://doi.org/10.1016/j.semcan.2020.08.009>

Received 11 August 2020; Received in revised form 17 August 2020; Accepted 17 August 2020

Available online 25 August 2020

1044-579X/© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

expression status of hormonal receptors, such as the estrogen receptor (ER) and progesterone receptor (PR), as well as human epidermal growth factor receptor 2 (HER2) which is also known as ERBB2, and proliferation index (Ki-67). Based on this, BC is classified into five subtypes, including luminal ER positive (luminal A and luminal B), HER2 enriched, normal like and triple negative receptors (basal like) (Fig. 1) [6,7].

Additional molecular subtypes were recently identified to include claudin low and molecular apocrine. This molecular sub-classification has served as a guiding principle for the utility of targeted therapies such as poly ADP ribose polymerase (PARP) inhibitors, HER2-targeted agents (e.g., Trastuzumab) and endocrine therapy (e.g., Tamoxifen), leading to better outcomes and management of BC [6].

Anticancer drug resistance is one of the major challenges in the management and treatment of advanced BC which can be caused by intrinsic and acquired factors that alter molecular/signaling pathways leading to poor survival [8]. Among these factors are tumor heterogeneity, genomic instability, self-renewing cancer stem cells (CSC), tumor microenvironment via direct interplay of extracellular matrix, growth factors, cytokines and stromal cells and epigenetic alterations/mutations that lead to metabolic variations (Fig. 2) [9–12].

Epigenetic modifications are an area of major interest as they play a role in overexpression of oncogenes or silencing of tumor suppressor genes, consequently stimulating tumorigenic pathways and affecting therapeutics in BC [13,14]. In this review, we aim to cover the general mechanisms of epigenetics, epigenetic deregulation in BC, drug resistance, association of epigenetics with poor clinical outcome and role of epigenetic biomarkers in diagnostics and therapeutics.

2. Epigenetic mechanisms

Epigenetics is a heritable molecular mechanism, controlled by external factors, that regulates genes expression without altering the actual sequence of DNA [15]. Progression of BC involves the accretion of aberrant changes both at genetic and epigenetic levels which ultimately lead to tumorigenesis. Therefore, epigenetic regulations caused by DNA methylation, histone modification, nucleosome remodeling, and RNA-mediated gene targeting, are known to modulate a number of molecular, cellular and biological pathways associated with breast carcinogenesis [16]. Recent findings indicate the role of epigenetic

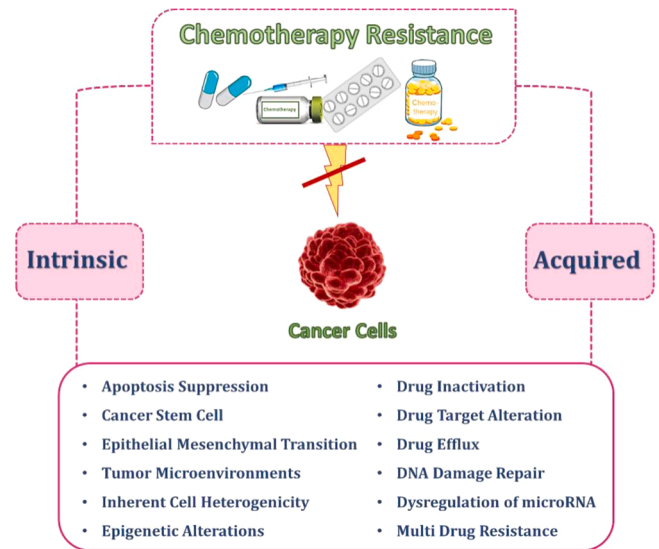


Fig. 2. Chemoresistance in cancer.

deregulations in BC hallmarks including drug resistance and stemness features [17]. Herein we elucidate the main molecular mechanisms of epigenetics and how epigenetic changes contribute into BC pathogenesis including the genetic reprogramming of oncogenes and tumor suppressor genes.

2.1. DNA methylation

DNA methylation is a critical enzyme-driven chemical modification where a methyl group is added covalently to cytosine or adenine in DNA sequence by a family of DNA methyltransferases (DNMTs) enzymes (DNMT1, DNMT3A, and DNMT3B) [18]. The already methylated DNA is maintained by DNMT1, while *de novo* methylation is carried out by DNMT3A and DNMT3B which target unmethylated and/or semi methylated CpG sites [19]. DNA methylation controls significant processes including transcription, post transcription, post translation, remodeling of chromatin, *imprinting* of genome, inactivation of X-chromosome and suppression of repeated elements of DNA [20,21].

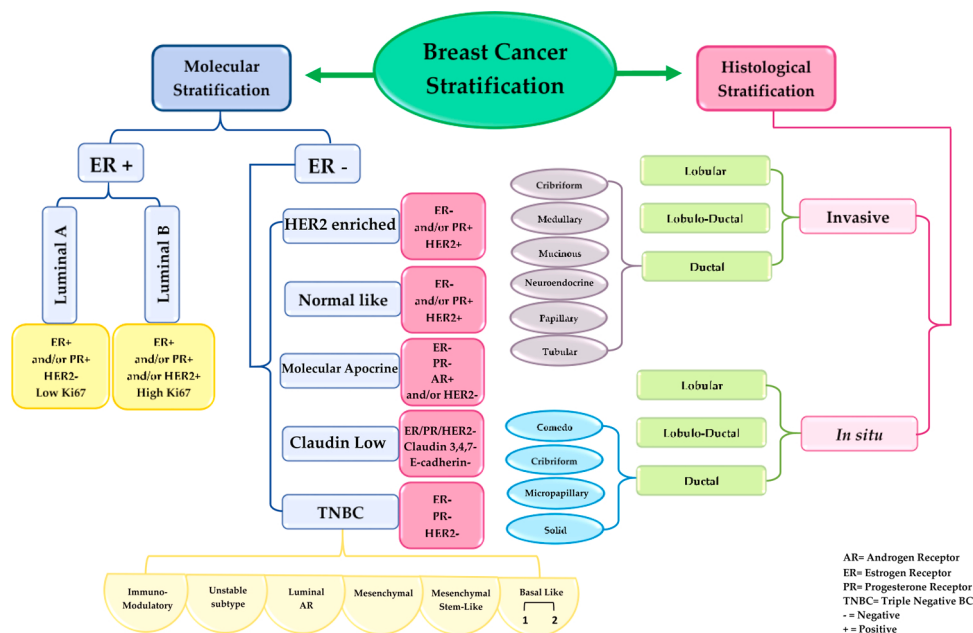


Fig. 1. Breast cancer classification.

As a result of methylation, certain gene regulator proteins are explicitly attached to DNA and restrict the transcription factors from accessing to chromatin which affects gene expression. Once the regulator sequences in the genes are altered, the transcription factors will no longer be able to identify them. Additionally, DNA methylation sets up a closed and restrictive chromatin form, making the modified chromatin unresponsive to nuclease digestion leading to reduced acetylation of histone proteins on the chromatin. Contrarily, intragenic regions, which control elongation of transcription and alternative splicing, have been found to have enhanced DNA methylation [22].

In vertebrate genome, CpG-rich regions, known as CpG islands, such as promoter regions, transcription start sites, and repetitive sequences are not generally methylated. Most of the genome is not GC rich and subsequently is highly methylated which is required for chromosomal stability [23]. In this way, hypomethylation and hypermethylation can happen simultaneously relying on the genome region and can thus influence the disease outcomes. Genome wide loss of DNA methylation i.e. hypomethylation is reported in various tumors and has been reported to have an impact on genome stability, DNA damage, and rejuvenation of retroviruses/transposons [20,24,25].

An aberrant DNA methylation, caused by endogenous and exogenous mutagenic processes, usually occurs in the CpG-rich regions of gene promoters contributing to the expression of proto-oncogenes or silencing of tumor suppressor genes (Fig. 3) [26]. Hence, carcinogenesis and metastasis are associated with loss of methylation in proto-oncogenes and turning on of transposable elements [27,28].

Furthermore, genome wide loss of methylation may cause loss of *imprinting* which plays a role in the early stages of transformation and tumorigenesis. For instance, insulin-like growth factor-2 (IGF2) plays a role in cell growth while loss of *imprinting* in IGF2 results in upregulation and instability of genome wide chromatin [29]. On the other hand, progression to malignancy and DNA hypomethylation are frequently related to mutations in DNMTs [30]. Downregulation of tumor suppressor genes such as BCL2, BRCA1, RAS and hypermethylation occurs in numerous neoplastic cells thus boosting malignant transformation [31].

Dysregulated methylation of genes and regulatory proteins has now become more evident in the pathogenesis of human cancers including BC. Accordingly, methylation-analysis assays are currently used in research aiming to develop novel diagnostic and therapeutic strategies of BC as evidenced in various studies [32–34]. There have been various underlying mechanisms explored as to how DNA methylation triggers cancer pathogenesis. For instance; the hypomethylation of SEPTIN7, TRIM27, LIMD2 and LDHA, have been associated with BC metastasis,

invasion and proliferation [33]. Also, it has been reported that APC, RARB, GSTP1, DAPK, and SFN genes are frequently methylated in BC cases [34]. Moreover, methylation induced aberrant expression of Claudin-6 (CLDN6) triggers breast carcinogenesis by recruiting MeCP2, deacetylating H3 and H4, and altering chromatin structure [35].

Dysregulated methylation of DNA is an important reversible epigenetic mechanism associated with BC pathogenesis via deregulated expression of genes. These genes are critical in the development of clinicopathological features such as tumor stage, histological grade, and TP53 status [36]. There are a number of reports showing how deregulated DNA methylation triggers altered gene expression converging towards the development of clinicopathological features of BC and hence have great diagnostic and therapeutic potential [37–39]. For instance, the epigenetic silencing of SFRP1 has been directly linked with poor prognosis in BC [36].

It has been observed that menopause accelerates epigenetic age-related diseases including cancer. Recently, a methylome based study reported that the accumulation of DNA methylation increased the susceptibility to develop postmenopausal BC. This underlines the importance of using these alterations as diagnostic biomarkers [40].

Aberrant epigenetic modifications of antioxidant gene expression have also been well studied and show an association with BC development and therapeutic challenges. Griess et al., reported a negative correlation of promoter DNA methylation and down-regulation of superoxide dismutase 3 (SOD3) expression in BC. The low expression/deletion of SOD3 gene is associated with more aggressive subtypes (TNBC and Her2+) and consequently poor clinical outcome in BC patients [41]. Hence, epigenetic silencing of SOD3 caused by differential methylation of CpG sites of the SOD3 gene may serve as a foundation for the use of epigenetic modifiers molecules in novel anti-cancer therapy strategies.

It is well documented that TNBC have widespread genome-wide hypomethylation compared to other BC subtypes. In 2018, Good et al. showed that the expression of Ten-eleven translocation methylcytosine dioxygenase 1 (TET1), DNA demethylase enzyme, is associated with a poor prognosis in TNBC. Additionally, TET1 is an oncogene that promotes oncogenesis through its abnormal hypomethylation by activating various signaling pathways including PI3K-mTOR. Hence, this may identify TET1 as a potential therapeutic target for TNBC [42]. Another study reported that the differential methylation status, gene expression and pathways activation are associated with the development of chemotherapy resistance to docetaxel in TNBC [43].

Noteworthy, aberrant DNA methylation is also critical in cancer stemness features. Recently, a comprehensive genome-wide analysis of DNA-methylation demonstrated that clustering of circulating tumor cells (CTCs) induces metastasis and progression in BC. This is caused by deregulated methylation binding sites for stemness and proliferation-associated transcription factors including OCT4, NANOG, SOX2, and SIN3A. This indicates that cluster-targeting has potential to inhibit metastasis and thus may be of therapeutic importance [44]. Furthermore, deregulated DNA methylation of homeobox C8 (HOXC8) gene, a master regulator of cell fate during embryonic development, reduces its expression in BC stem/progenitor cells and promotes stemness features [45]. Worner et al. suggested that deregulated DNA methylation is one of the critical underlying events associated with transformation of the mesenchymal stem cells into tumor-forming cells in BC development [46].

It has also been shown that DNA methylation and other epigenetic processes play a vital role in regulating the expression and functioning of non-coding RNAs (ncRNAs), which are critical in maintaining the biological homeostasis. Alterations in these mechanisms lead to aberrant expression of ncRNA favoring BC pathogenesis. Interestingly, a recent study by Shi et al. investigated the epigenetic silenced miR-133a-3p and reported its correlation with BC metastasis and stemness features via upregulating mastermind-like transcriptional coactivator 1 (MAML1) [47]. Also, it has been revealed that the aberrant DNA methylation of the

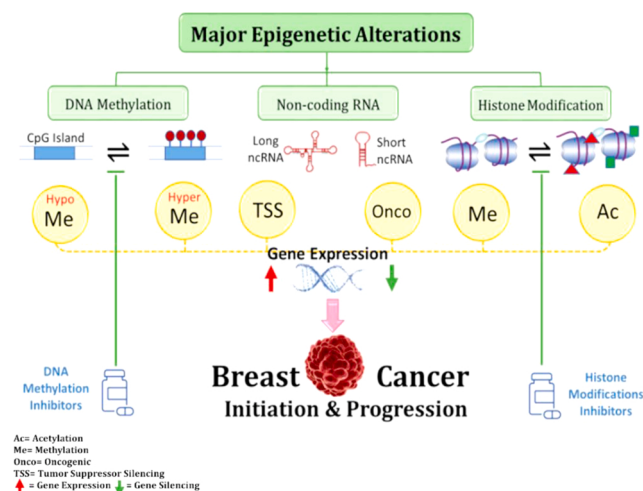


Fig. 3. Schematic showing methylation of CpG island and histone modifications causing deregulation of oncogenes and tumor suppressor genes.

tumor suppressor microRNA-874 promotes breast carcinogenesis and is associated with lymph node metastasis [48]. Another investigation revealed that aberrant DNA methylation lead to BC pathogenesis via dysregulation of 12 ncRNAs including; miRNA124, 125b, 127, 132, 137, 148a, 191, 193a, 203, 34b, 375, 9 [49]. Thus, these alterations might serve as a prognostic biomarker, and therapy targets.

Drug resistance in BC cells due to reprogramming of epigenetic and genetic regulatory mechanisms poses a huge challenge for effective cancer therapy. Here we have included some of the recent findings on how deregulated methylation status of various genes related to cell growth and survival converge towards drug resistance in BC. An interesting finding revealed that remodeling and reprogramming of 3D epigenome are the central regulatory underlying mechanisms of endocrine resistance in ER + BC. This is due to aberrant methylation along with differential ER-bound enhancer – promoter interactions [50].

Enhancer of zeste homolog 2 (EZH2), an oncogenic histone methyltransferase, has a well-established role in the progression of aggressive cancers including BC. EZH2 has a critical role in connecting two critical epigenetic programs, it interacts directly with DNA methyltransferases and control DNA methylation functionally. Also, EZH2 is reported to be aberrantly activated in various forms of cancer including BC. The expression of EZH2 increased with various stages: lower in normal, and increased in atypia, ductal carcinoma in situ, invasive and metastatic BC samples [51,52]. Wherein, more aggressive BC and poor clinical outcome have been found to be associated with elevated EZH2 expression [51]. It has also been shown that Tamoxifen resistance in BC cells is driven by epigenetic reprogramming as a result of aberrant expression of EZH2 mediated the silencing of the ER α cofactor GREB1 expression through DNA methylation [53].

Furthermore, deregulation of DNA methylation mediated trastuzumab resistance in HER2 + BC via epigenetic reprogramming and suppression of TGFBI, CXCL2, and SLC38A1 genes suggesting that promoter hypermethylation of these genes could be of great therapeutic importance for HER2 + BC patients [54]. Inactivation of Spalt-like transcription factor 2 (SALL2) as a result of aberrant DNA methylation leads to tamoxifen resistance in BC via downregulation of ER α and PTEN. Thus, the use of DNMT inhibitor induces SALL2 upregulation to overcome tamoxifen resistance in BC cells which indicates the importance of co-therapy leading towards a better clinical outcome (Fig. 4) [55].

Metabolic reprogramming, an important cancer hallmark is another major challenge, maintained by a number of signaling regulatory circuits affected and controlled by aberrant methylation or epigenetics. It was recently discovered that methylation mediated metabolic reprogramming of a key glycolytic enzyme pyruvate kinase M2 (PKM2) by co-activator-associated arginine methyltransferase enhances BC cell energy, proliferation, migration and metastasis [56].

Increasing evidence supported the role of DNA methylation and histone modifications in the progression of cancer as well as its role in chemoresistance which has garnered lot of attention [57]. Silencing of regulatory genes through hypermethylation supports uncontrolled cancer cell growth whereas hypomethylation causes activation of genes essential for metastasis and chemoresistance (Fig. 4) [58]. Various genes known to be involved in process of metabolizing drugs, repairing the cellular damage induced either by themselves or through any agents, play a pivotal role in drug resistance development [59]. For instance, Chekhun et al., 2007 analyzed the hypo- and hypermethylated DNA sequences and identified dysfunctional genes sequence involved in estrogen metabolism, apoptosis cell-cell contact and demonstrated that two opposing hypo- and hypermethylation processes may or may not enhance and complement each other in the disruption of pathways [60].

Poor or adverse survival outcomes have been found to be associated with DNMTs, histone lysine methyltransferases (HKMTs), protein arginine methyltransferases (PRMTs) PRMT 1, 3, 5, 7, 8, and histone lysine demethylase 2A (KDM2A) in BC (Fig. 4) [61–63]. Additionally, genome-wide analysis for breast tumor and adjacent tissues found increased levels of DNA methylation in ductal carcinoma to be related to

Reversal of Epigenetics

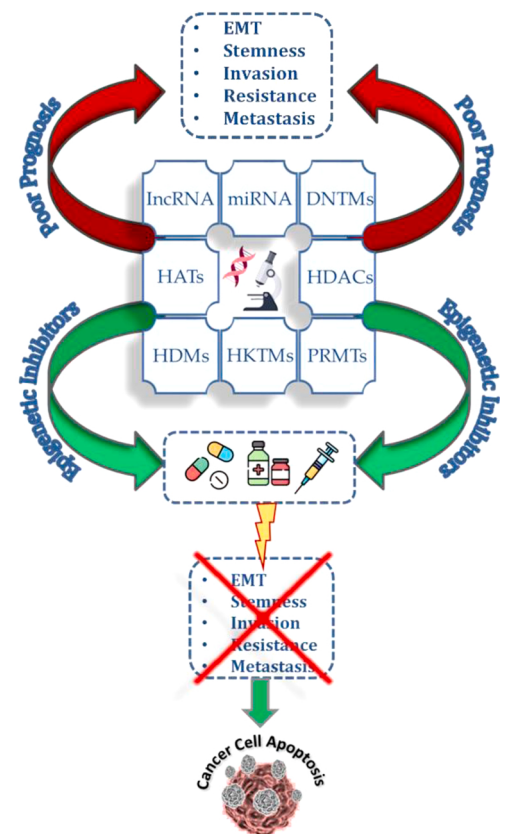


Fig. 4. Reversal of Epigenetics. The figure illustrates the studying of reversing the epigenetic alterations associated with poor clinical outcomes in BC using potential epigenetic biomarker and novel therapeutic pathways may lead to a successful anti-cancer treatment.

the invasive form of BC and metastasis [64]. Hypermethylation of BRCA1 has been found to be associated with ER- BC and poor clinical outcomes [65]. Also, the epigenetic silencing of MSH2 through the hypermethylation of a promoter induced doxorubicin resistance in BC cells. Being reversible, these alterations may serve as targets to develop epigenetic therapies to re-sensitize doxorubicin-resistant BC cells (Fig. 4) [66]. A contrary relationship has been shown between methylation of the ER β gene and tamoxifen resistance. Overall, there was denser methylation in resistant tumors compared to control tumors [67].

Epigenetic aberrations in tumor microenvironment (TME) have been reported in BC with implications of poor clinical outcome and drug resistance (Fig. 2). A study on AU565 and SKBR3, breast cancer cell lines, showed that CAF/Stromal secreted factors (such as cytokines, MMPs, and growth factors, TGF- β , miRNAs etc.) are actively involved in epigenetic pathways with subsequent upregulation of specific genes via DNA methylation patterns. This leads to reprogramming of cancer cell response to the TME locking in transcriptional changes that initiate them [68].

On the other hand, a study on the influence of epigenome on PI3K signaling pathway reported that epigenetic regulator (KMT2D) is a key factor that leads to the inhibition of PI3K pathway leading to subsequent activation of ER dependent transcription. As such, this epigenetic change has been associated with higher tumor size reduction in mice models indicating the utility of epigenetic therapy in PIK3CA-mutant, ER-positive BC patients [69].

A study on the characterization of specific DNA methylation profile in HER2 BCs observed a strong association between ER and PR gene methylation and expression [70]. The study postulated that HER2+ cancers created an environment that induced PGR and HSD17B4 methylation leading to low levels of functional ER and 17- β -estradiol

metabolizing enzymes thus affecting anti-tumor activity of tamoxifen and producing a resistant phenotype. In addition to this, the study also reported that DNA methylation changes were apparent in the stroma of HER2+ cancers indicating the involvement of epigenetic imprints within the environment that facilitate tumor progression [71]. Another large-scale study observed distinct epigenetic changes in the microenvironment (epithelial, myoepithelial cells and stromal fibroblasts) of both normal breast tissues, in situ and invasive breast carcinomas. This indicates that epigenetic imprints in the microenvironment may drive aggressiveness and resistance in BC [72].

N⁶-methyladenosine (m⁶A) is the most prevalent deregulated methylation detected in the aberrant expression of cancer associated genes, drug resistance and stemness via modulating signaling mechanisms such as BRD4, MYC, SOCS2 and EGFR [73]. It was observed that m⁶A triggers BC pathogenesis via targeting apoptotic regulatory genes [74]. The over-expression of FTO (Fat mass and obesity-associated protein or alpha-ketoglutarate-dependent dioxygenase), a key m⁶A demethylase, triggers breast carcinogenesis by targeting BNIP3, a pro-apoptosis gene and tumor suppressor. Recently it has been shown a remarkable decreased m⁶A methylases (METTL3, METTL14 and WTAP) expression with a concomitant over expression of FTO in BC samples [75].

Epigenetic modifications of stemness features of CSCs are often associated with disease progression and therapeutic failure. Hypoxia induced changes in methylation status, it induces ALKBH5 mediated demethylation and stabilization of NANOG, KLF4 mRNA crucial stemness proteins, leading to stemness of BC [76,77]. This, critical growth and migration mechanism, indicates the critical role of epigenetic alterations due to m⁶A in progression of BC, hence m⁶A associated targets may be of great therapeutic importance for BC [78,79].

2.2. Histone modifications

Histone codes are referred to post translational changes in histone proteins. Changes in histone proteins introduce an additional level of multifaceted nature to phenotypes in cell [80]. Histone proteins are key elements of the nucleosome, which are accountable for keeping repressive chromatin in stable form. Histones are exceptionally alkaline, so they firmly bind with DNA, which is negatively charged by salt bridges and hydrogen bonds. The nucleosome is made of an octameric core having duplicate copies each of H2A, H2B, H3 and H4 histones wrapped by strands of DNA and a H1 linker histone. Repeating subunits of nucleosomes produce chromatin, which can possibly characterize the state in which hereditary data is organized inside a cell. Changes in conformational structure of chromatin present a specific positioning of the genome, in a dense or non-dense condition that regulates gene expression [81]. The structure of chromatin is changed by histone post translational modification, this caused by addition of chemical groups to the N-terminal tails. The charge characteristics of histones are influenced by further groups added; as a result the structure of dense nucleosome is relaxed or closed.

The above-mentioned moieties have the ability to bait more proteins which precisely identify the altered residues. As a result of this, environment of the chromatin is changed, due to which the access to the cis-regulatory elements is more restricted or relaxed.

Alterations of histones can have enormous impact on processes related with DNA such as packaging, recombination, repair, replication, and transcription regulation. The most widely recognized modifications are methylation and acetylation, which mostly happen close to promoter and enhancer regions [82]. These changes are regulated by numerous enzymes, for example, histone methyltransferases, demethylases, acetyltransferases, and deacetylases [83]. Other histone modifications include ubiquitination, phosphorylation, and other uncommon ones such as ADPrubosylation, citrullination, formylation, deamination, propionylation, O-GlcNAcylation, butyrylation, proline isomerization, and crotonylation (Fig. 5) [84]. Changes at the chromatin level due to errors

in post-translational changes in histone are called epimutations which may change gene expression patterns and give rise to a disease [85].

It has been reported that histone acetylation modifications (HAMs) play significant role in BC tumorigenesis. Recent studies on aberrant HAMs aimed to reveal the initial molecular processes involved in the evolution of BC prognosis and treatment (Fig. 4) [86]. Xi et al. (2018), profiled histone modifications in BC using cell lines representing the five main molecular subtypes of BC. This study generated data defined subtype specific chromatin signatures which can serve as a reservoir for histone modification profiles in BC to nominate potential biomarkers with the possibility to find new personalized and targeted therapeutic for BC (Fig. 4) [87].

Elshikh et al. investigated 880 human BC samples and documented that the differential levels of lysine acetylation (H3K9ac, H3K18ac, and H4K12ac), lysine (H3K4me2 and H4K20me3), and arginine methylation (H4R3me2) were observed in poorer prognostic BC subtypes, including basal and HER2 + . Whereas hypoacetylation of H4K16ac is correlated with better clinical prognosis. This suggests the use of these modifications as prognostic and indicative markers for BC (Fig. 5) [88].

Aberrant acetylation status is another critical epigenetic event related to reprogramming and modulation of gene expression implicated in BC pathogenesis, stemness metabolic reprogramming and resistance to therapeutics. Chemotherapy resistance is posing as one of the major clinical challenges in the management of BC. In line with this, an interesting finding shows that the accumulation of acetylated mitochondrial superoxide dismutase (SOD2) and mitochondrial reactive oxygen species (mtROS) enhance stem cell reprogramming in late stage of BC via promoting hypoxic signaling of hypoxia-induced factor 2α (HIF2α). Also, SOD2 acetylation provides BC cells with the ability to develop resistance against endocrine therapy (tamoxifen) via increasing peroxidase activity which is a well-established characteristic of CSC. This suggests that the acetylation of SOD2 might contribute by playing an effective role in more invasive, drug resistance and poor outcomes [89].

Recent study findings showed that acetylation of the serine-arginine protein kinase 1 (SRPK1) is a key factor in the development of cisplatin resistance in BC cells. This may serve as a potential therapeutic opportunity to overcome the platinum related drug resistance [90].

Altered epigenetic changes via histone modifications molecules are critical in the pathogenesis and treatment of BC. Chatterjee et al. have reported that the use of resveratrol, a natural anticancer agent, restores the expression of tumor suppressors by modulating epigenetic changes due to both methylation and acetylation at promoter of histone in BC cells [91].

Deregulated chromatin alterations by histone modifiers modulate the expression of multiple genes associated with oncogenesis and development of cancer stemness features. Recently, it has been delineated that use of HDAC inhibitors suppressed the cancer stemness features in BC via inhibiting expression of super-enhancers (SEs) associated oncogenes [92].

Generally, there are 18 potential enzymes of histone deacetylases (HDACs) grouped into four classes. In which HDAC class I (HDAC 1, 2, 3 and 8) seem to be the most important ones in carcinogenesis [93]. The use of HDAC inhibitors (HDACis) has showed promising outcomes in the attenuation of drug resistance in BC cells via targeting key efflux transporters, multidrug resistance protein 1 (MDR 1, ABCB1, P-glycoprotein) and BC resistance protein (BCRP, ABCG2) (Fig. 4) [94]. Worth mentioning, another study reported the role of epigenetic alterations as a prime cause of radio-resistance in BC cells. This caused by altered activities, high HDAC and low histone acetyl transferase (HAT), leading to suppressed or loss of histone phospho-acetylation and chromatin condensation. The variation of HDAC activity among BC patients suggests the implementation of a prior assessment of patients' epigenome to maximize the benefit of HDAC inhibitor-based radio-sensitization [95].

The expression of HDAC 1 and HDAC 6 have been studied in BC subtypes and show that the highest expression was observed in luminal

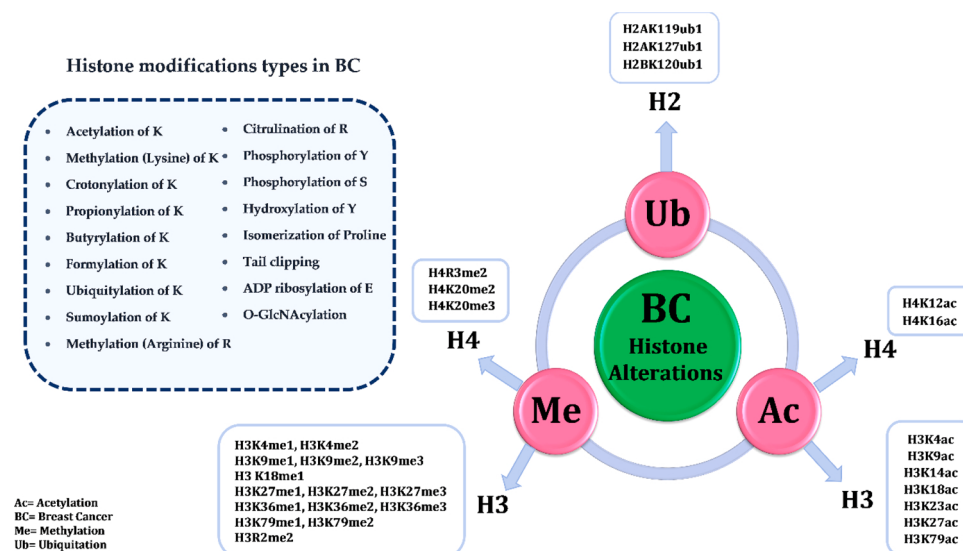


Fig. 5. Histone modifications types and certain profiled Histone modifications in BC.

A and Luminal B subtypes respectively [96,97]. Thus, the expression of HDAC 1 and 6 are good prognostic factors and are positively associated with better therapeutic outcomes in ER + BC [98]. Whereas the higher expression of class I HDAC2 and 3 were associated with highly aggressive (ER-/PR-) BC subtypes. Moreover, declined survival in ER + BC subtype has been associated with the elevated expression of class II a HDACs [99]. Lapiere et al. revealed that a significantly high expression of class II a (HDAC9) in basal subtype of BC was associated with the expression of SOX9 and poor prognosis of BC [100].

Lysine-specific demethylase 1 (LSD1), a histone methylation eraser, is highly expressed in BC acts on H3K4 and H3K9 [101,102]. In two different studies, it has been reported that the LSD1 expression is directly correlated with the progression of BC and was found to be highly expressed in ER-/PR- BC subtypes [102,103]. Similarly, EZH2 is reported to be aberrantly activated in various forms of cancer including breast cancer and is associated with aggressive form of breast cancer [51]. The reduction of CAF related histone mark, H3K27me3, leading to decreased expression of methyltransferase (EZH2) and subsequent upregulation of thrombospondin type 1 motif 1 has been associated with tumor invasiveness in BC [104].

Although tumorigenic role of histone proteins alterations is well discussed, adding to this it has recently been explored that interaction of pygopus 2 (Pygo2), a co-activator of Wnt/ β -catenin signaling, with bi- or trimethylated lysine 4 of histone-3 is critical for BC development and metastasis and thus interfering pygo2- H3K4me2/3 interaction could be an important therapeutic option in BC management [105]. Furthermore, epigenetic alterations due to deregulated expression of LSD1 are also associated with reprogramming in BC stem cells stemness features [106]. Interestingly, a recent study shows that histone demethylase KDM7A, is critical for the growth and maintenance of BCSCs via upregulating the stemness-associated factors KLF4, c-MYC and BCL2 [107].

Epigenetic reprogramming affects epithelial-mesenchymal transition (EMT), critical for cancer metastasis and drug resistance, through modulating the HDACs, TET2 hydroxylase along with Mbd3/NuRD complex eventually making cancer cells in a highly metastatic mesenchymal state and hence suggesting combinatorial interference may be efficient in suppressing BC metastasis [108].

Increased expression of Nicotinamide N-methyltransferase (NNMT) is often correlated with poor clinical outcome and resistance in BC patients. The underlying mechanism includes stabilization of SIRT1, a deacetylation enzyme and its inhibition overcome resistance to adriamycin and paclitaxel in BC cells [109]. Poor prognosis in BC has been shown to be associated with the expression of histone acetyltransferases

(GTF3C4 and NCOA3) [61]. Additionally, it has been shown that in BC cells, p300/CBP (CREB binding protein), which is a transcriptional coactivator of BRCA1, facilitate crosstalk between ER and NF- κ B signaling pathways [110]. Moreover, it epigenetically induces EMT in breast metastasis by cooperating with DOT1L-cMyc complex. The acquisition of cancer stem cell-like properties in breast carcinogenesis is associated with the elevated level of p300-DOT1L-cMyc [111].

2.3. Noncoding RNA processing

In eukaryotic cells, a large portion of the genome is transcribed but not translated. It is well known that 2–3 % codes for proteins while 80 % is non-coding RNA [112,113]. Non-coding RNAs can be categorized into small and long non-coding RNAs based on their molecular lengths. Small non-coding RNAs are less than 200 nucleotides and may further classified into microRNA, piwi-interacting RNA, small nuclear RNA, and small-interfering RNA. The best described small non-coding RNAs in cancers are microRNAs, which obstruct protein syntheses either by cleaving mRNA or inhibition of translation [114].

The non-coding RNAs whose length is more than 200 nucleotides are categorized as long non-coding RNAs (lncRNAs). They control expression of gene both in cis and trans mechanisms. In cis mechanism, they are located in vicinity of target gene in the genome to repress gene expression by transcriptional interference in which the initiation of adjacent transcription is suppressed due to elongation of lncRNAs transcript [115]. Also, lncRNAs have the ability to control the expression of gene in cis by attaching close to regulator DNA sequences and causing either to break preinitiation complex or overlay chromatin region [116, 117]. In trans mechanism, the lncRNAs control gene expression by interacting with epigenetic regulators, transcription factors, and RNA polymerases in which they may change localization or enzymatic functions of proteins [118–120].

Recently, non-coding RNAs (ncRNAs) were reported for their contribution in a number of epigenetic processes controlling gene expression such as regulation of transcription, post transcriptional modification, and modulation of chromatin structure [121]. Current studies are focusing on the role of ncRNA in BC [122–125]. Recently, a study described and confirmed six lncRNAs markers in luminal BC subtype that remarkably enhanced its prognosis and possible therapeutic aims [126]. Another study investigated the role of FLVCR1-AS1 lnc RNA in BC and reported its role in tumorigenesis process with its value as a possible therapeutic target [127].

It has been reported that tumor invasiveness in BC is associated with

the high expression of lncRNA HOTAIR [128,129]. It has been shown that lncRNA-ATB activated by TGF- β induced Trastuzumab resistance in BC cells by upregulating ZEB1 and ZNF-217 and competitively binding miR-200c to induce EMT [130]. It has also reported that paclitaxel resistance is induced due to the high expression of lncRNA H19 leading to inhibit the transcription of pro-apoptotic genes BIK and NOXA [131].

Recently it was shown that lncRNA DANCER (differentiation antagonizing nonprotein coding RNA) has role in inflammatory BC related phenomenon: inflammation-mediated EMT, and cancer stemness in late-stage TNBC. The investigators also showed that SOCS3 was down-regulated by lncRNA DANCER with the help of EZH2 epigenetic mechanism [132]. It has also been shown that lnc RNA cancer susceptibility candidate 9 (CASC9) binds to EZH2 and regulate the MDR1 gene to result in drug-resistant BC [133].

Micro-RNA controls expressions of various genes either through suppression of the translational process or through degradation process. For instance, chemosensitivity of MCF-7 cells towards doxorubicin were increased by downregulating MDR-1 by miR-451 and MRP-1 by miR-326 [134]. Ectopic expressions of miRNA 221 and miRNA 222 by inhibiting p27 (Kip1) transformed MCF-7 cells from hormone sensitive to a resistant cell line thus indicating that miRNAs supports growth of cancer cells even in absence of estrogen and support resistance towards endocrine therapy [135]. Similarly, miR-873, Let-7b/Let-7i also rendered resistance to tamoxifen treatment through inhibiting ER α and p27Kip1 [136]. MiR-129–5p by modulating EMT and through inhibition of ATP-binding cassette subfamily B member 1 (ABCB1), MDR can be reversed [137]. Similarly, by suppression TUBB3, ZEB1 and ZEB2 by miR-200c cancer cells were chemosensitized to paclitaxel treatment [138]. Whereas through downregulating Bcl-2 antagonist killer 1 (Bak1), MiR-125b supported drug resistance to paclitaxel [139]. Targeting of BRCA1 helped miR-218 to increase sensitivity of breast cancer cells towards cisplatin [136,140].

CAF secreted soluble factors are also known to activate growth factor dependent-MAPK signaling in BC. As such, changes in these signaling pathways can also manipulate the expression of microRNAs (miRNAs). A study on BC was able to identify a novel CAF secreted miRNA signature known as hMAPK-miRNAs miR-221/222. This hMAPK-miRNA signature was observed to induce ER repression in ER-positive cell lines via paracrine interactions within the tumor microenvironment leading to poor outcomes and survival [141].

3. Recent research findings converge on promising diagnostic and therapeutic role of epigenetics in breast cancer

BC is the most prevalent cancer in females with high morbidity and mortality rates worldwide. Within the last decade, the rates have jumped by more than 20 % and 14 % respectively [142]. Mammography is a gold-standard screening tool for BC diagnosis; however, it has significant limitations due to the lack of sensitivity and specificity in BC size of < 1 cm which lead to misdiagnosis, overdiagnosis and/or overtreatment [143]. Successful management of this disease is based on the early detection of BC patients followed by a targeted treatment which can improve the 5-year survival rate by up to >93 %. Consequently, an inaccurate diagnosis affects the patient negatively and results in unfavorable clinical outcomes.

Currently, an early diagnosis of BC remains one of the greatest challenges. This highlights the need for the development and establishment of a robust and accurate diagnostic tool to screen, detect and monitor the progression of this disease [144]. Thus, establishing novel diagnostic and prognostic biomarkers will facilitate the early detection of this disease which provides better opportunities in the prevention and management of BC, leading to a major shift in the reduction of mortality and morbidity of BC worldwide [145].

Herein, we focus on the current state of this discipline and emphasize the role of epigenetics as potential biomarkers for detection, prognostication and/or prediction of BC treatment efficacy. This section also

reviews a crucial element of future targeted cancer therapy by describing the potential use of epigenetic modifiers in the prevention and treatment of BC.

3.1. Significance of using epigenetic alterations as diagnostic, prognostic, and predictive biomarkers in Breast Cancer

A biomarker is a measurable biochemical particle that can be found in tissues, blood, or body fluids in response to disease development and progression. This includes DNA, RNA, protein, or an epigenetic modification. An accurate tumor biomarker indicates the presence, assesses prognosis, and even guides targeted therapy of cancer [146].

Carcinogenesis is a complex multistep process involving both genetic and epigenetic changes that generate multiple changes in gene expression which lead to an altered regulation of the cell cycle [147]. Epigenetic alteration, such as aberrant DNA methylation and histone acetylation at the promoter regions of genes, is one of the initial events in the cancer inducing mechanism as it contributes to the silencing of distinct genes (such as proapoptotic, cell cycle-inhibitor or DNA repair genes). It has been reported that the number of aberrantly methylated genes identified in BC is increasing rapidly [148]. Aberrant DNA methylation is considered as an attractive biomarker to be examined in liquid biopsies for many reasons; its early onset, cancer specificity, biological stability, and availability in bodily fluids. Being relatively highly stable and detectable in circulating cell-free tumor DNA (ccfDNA) from liquid biopsies, this enables the possibility of implementing DNA methylation as a fast, reliable, cost-effective and non-invasive testing of BC [146,149–151].

It is well evident that aberrant DNA methylation plays a key role in breast tumorigenesis and drug resistance. Furthermore, it has been shown that the alteration of the DNA methylation profile of BC patient blood arises years before the cancer is clinically detected [152]. Consequently, aberrant DNA methylation has the potential to constitute as a valuable biomarker for BC [153].

As shown in Table 1, several studies have been conducted towards uncovering accurate epigenetic based biomarkers with high sensitivity and specificity. For instance, hypermethylation of hyaluronoglucosaminidase 2 (HYAL2) in blood can be detected in the very early stage of BC cases. This suggests that the HYAL2 methylation level can be used as an early marker to detect BC with great sensitivity and specificity of 64 % and 90 % respectively [154]. On the other hand, secretoglobin family 3A member 1 (SCGB3A1) did not distinguish cancerous cases from controls [151].

Due to the inter/intratumor heterogeneity of BC, it has been indicated that the use of one epigenetic biomarker for the detection of BC might be specific for one subtype and possibly will not serve for another which leads to false diagnosis. Consequently, a couple of gene panels were developed and evaluated to improve the sensitivity of BC detection. For instance, a two gene panel, RAR β and RASSF1A, was assessed by Kim et al., 2010 which reported the detection of BC with a significant sensitivity and specificity of 94.1 % and 88.8 % respectively [155]. Similarly, a six-methylated-gene panel consisting of (SFN, P16, hMLH1, HOXD13, PCDHGB7 and RASSF1A) and three gene panel (APC, FOXA1 & RASSF1A) were able to detect BC in serum with a high level of sensitivity and specificity [156,151].

Additionally, novel DNA methylation markers, PRAC2, TDR10 and TMEM132C, were identified as potential diagnostic and prognostic markers due to their high expression in breast tumor tissue specifically in ER-positive patients [157]. Moreover, Nandy et al. proposed the use of five panel histone epigenetic biomarker (APLF, HJURP, MacroH2A.1, γ H2AX, & H2Bub1) to serve as a potential prognostic biomarker to detect the probability of developing metastasis of BC [158].

Epigenetic characteristics of BC can also be determined using ctDNA analysis for early detection and targeted therapy of BC [159]. Agostini and colleagues reported identifying the ALU247 methylation in BC patients using the MethyLight® method with greater than 99 % sensitivity

Table 1

The most studied epigenetic markers as potential biomarkers with high specificity and sensitivity.

Potential biomarker	Category	function	sample type	role	Sens. (%)	Spec. (%)	Reference
RAR β , RASSF1A	Panel marker	Diagnostic	Serum	Detect <i>in situ</i> & invasive ductal BC	94.1	88.8	[155]
ALU247	Single marker	Diagnostic	Plasma	Detect metastatic BC	> 99	69	[160]
FHIT	Single marker	Diagnostic	Serum	Early diagnosis of ductal BC	Significant	Significant	[161]
APC, RAR β	Single marker	Diagnostic	Serum	Early diagnosis of ductal TNBC	93.4 95.6	95.4 92.4	[170]
HYAL2	Single marker	Diagnostic	Peripheral Blood (Leukocyte)	Diagnose of an early stage of BC	64	90	[154]
SFN, P16, hMLH1, HOXD13, PCDHGB7 & RASSF1A	Panel marker	Diagnostic	Serum	Detection and monitoring of BC patients	82.4	78.1	[156]
APC, FOXA1, & RASSF1A	Panel marker	Diagnostic	Plasma	Detection and monitoring of BC patients	81.82	76.92	[151]
ESR1	Single marker	Predictive	Peripheral Blood (CTCs)	Predict endocrine therapy efficacy in BC patients	Significant	Significant	[168]
KEAP1	Single marker	Prognostic & predictive	Tissue	Predict resistance to chemotherapy regimens involving taxanes	Significant	Significant	[163]
RASSF1, BRCA1, PITX2, CDH1, RAR β , PGR, PCDH10 + GSTP1, RASSF1, & RAR β	Single marker + Panel marker	Prognostic	Serum	Poor prognosis	Significant	Significant	[171]

and 69 % specificity [160]. Liu et al. examined the level of FHIT promoter methylation in serum and showed it was significantly associated with ductal breast carcinoma; this may be useful for the early diagnosis of this type of BC [161]. The three gene-panel of [Adenomatous polyposis coli (APC), Fork-head box A1 (FOXA1) and Ras association domain family 1 isoform A (RASSF1A)] hypermethylation in ccfDNA was able to identify BC with sensitivity, specificity and accuracy higher than 75 % [151].

The epigenetic biomarkers can be useful as predictive markers to predict therapeutic drug responses [162]. Examples include the methylation of KEAP1 gene which was linked with a better overall survival; this might serve as a biomarker that suggests resistance to chemotherapy regimens involving taxanes [163]. It has also been shown that p16 promoter hypermethylation in BC suggested that p16 may be used as a prognostic and predictive marker to predict treatment response to hormonal therapy [164]. Besides, hypermethylation of p16 is significantly linked with a candidate pre-cancerous hypermethylation profile (BRCA1, BRCA2, ER α , and RAR β 2). This suggests that p16 promoter hypermethylation of candidate genes could be detectable in early stages before pathological changes; this could be used to diagnose females who should be closely monitored for BC [165].

DNA methylation of ESR1 in plasma cctDNA samples is significantly linked with the lack of estrogen receptor (ER) expression in excised tumors which is associated with lack of response to endocrine treatment [166]. Consequently, ESR1 might serve as a potential predictive biomarker for endocrine treatment efficacy [167,168]. The association between BRCA1 hypermethylation and increased sensitivity to platinum-based chemotherapy in ovarian and BC can also be utilized to implement BRCA1 as a predictor response biomarker to platin-based chemotherapy in BC patients [161,169].

The blood-based test for BC biomarkers was approved by the Food and Drug Administration (FDA). Several cancer antigen biomarkers such as CA15–3, CA27.29, CA-125, CEA (carcinoembryonic antigen) and CTCs are exclusively recommended as prognostic markers to monitor treatment efficacy and disease relapse, rather than early diagnostic markers. Currently, mutation analysis screening test using gene mutation markers (BRCA1 and BRCA2) is the only used test for screening of hereditary BC [34]. Additionally, the currently used molecular In-Vitro Diagnostics (IVDs) include tumor profiling tools such as Prosigna, Mammprint, OncotypeDX, and Endopredict which are based on gene expression and mutational profiles using conventional tissue biopsies and not DNA methylation. Oncotype DX is the most used one and designed for patients with ER+/HER2- and LN- primary BC. EndoPredict

is a new predictive tool based on the analysis the expression of 8 targeted genes to estimate the risk of distant recurrence in BC patients with ER+/HER2- [172]. These cancer profiling tools were developed aiming to categorize BC patients into risk/treatment groups to assist in adjuvant treatment decision. However, their efficacy in clinical practice is limited to certain BC subtypes and therefore their implementation remains restricted.

Currently, DNA methylation markers are not yet implemented in the clinical setting of BC detection. However, the prognostic value of these markers was utilized to develop a reliable PCR based prognostic assay for BC. In 2018, Qiagen and Therawis introduced the first clinically validated DNA methylation-based assay, *therascreen*® PITX2 RGQ. This predictive IVD is available in Europe to predict the response of certain high-risk BC patients (ER+/HER2- and LN+) to anthracycline-based chemotherapy with or without endocrine therapy. Hence, the use of *therascreen*® PITX2 RGQ is limited as this test is not beneficial for patients with more aggressive and/or resistant subtypes such as HER2+, TNBC or BC with lymph node involvement [173]. Besides, IvyGene is a validated DNA methylation-based test in the USA which is used to detect early stage of four common cancers including BC (breast, colon, liver and lung). The use of a panel of 46 markers is able to quantify the presence of these cancers using blood samples from cancer suspected patients.

3.2. Utilizing epigenetic modifying drugs as a therapeutic approach in BC

As mentioned earlier, in cancer cells, gene alterations can result from both mutations and/or through epigenetic modifications to chromosomes that change gene expression patterns. Epigenetic modifications, unlike genetic mutations, include abnormal cytosine DNA methylation and histone hypoacetylation in the promoter region of important genes and are generally reversible. Hence, restoring normal growth phenotype is theoretically possible through implementing epigenetic modifying drugs to reverse aberrant epigenetic alterations and this appears to be a desirable target for cancer therapies [174,175].

Accumulating evidence suggests that epigenetic therapies could potentially work synergistically, when combined together and/or with conventional chemotherapy, in increasing therapeutic effects. The use of DNA methyltransferase (DNMT) and/or histone deacetylase (HDAC) inhibitors in BC treatment have been tested in various trials to evaluate the efficacy of these drugs to overcome epigenetic alterations and hormone resistance [176].

Molecules listed in Table 2 include some of the potential epigenetic

drugs for BC treatment including previously FDA approved and investigational epigenetic drugs (DNMT and HDAC inhibitors). For instance, azacitidine and decitabine (cytidine analogs), are approved DNMT inhibitors which can induce DNA demethylation. Also, vorinostat, panobinostat, belinostat, and romidepsin are FDA-approved HDAC inhibitors.

Several clinical studies have investigated using a combination of epigenetic modifiers (Table 2) and shown promising anticancer effects against breast carcinoma. They also reported positive results in favor of combined epigenetic drugs with/without anticancer therapy over the use of single-agent therapy. For instance, phase I and II clinical trials have been conducted using HDAC inhibitors (vorinostat, panobinostat and entinostat) alone or in combination with other therapeutic agents such as endocrine therapy, immunotherapy and/or chemotherapy [177]. The results from terminated or completed trials ranged from no response to 55 % response [176].

In 2016, Li and his colleagues implied that the extensive expression of histone deacetylase enzyme 5 (HDAC5) in human BC tissues indicates that HDAC5 may serve as a potential novel prognostic marker and selective therapeutic target for BC [178]. Histone deacetylase enzymes 1 and 3 (HDAC1 and HDAC3) are also highly expressed in BC. *in vitro* studies showed that the exposures of breast carcinoma cells to HDAC1 inhibitors (vorinostat or entinostat) reverse the immune evasion to enhance the sensitivity to T-cell-mediated lysis [179,180]. Moreover, several HDACis have indicated therapeutic effects against triple negative breast cancer (TNBC) such as vorinostat, sodium butyrate, mocetinostat, panobinostat, entinostat, YCW1 and N-(2-hydroxyphenyl)-2-propylpentanamide [181].

HDAC inhibitors (HDACis) have also shown limited effect as single agents. Conversely, in combination with other anticancer agents, HDACis demonstrated promising therapeutic results. For instance, LMK-235 is a promising new HDAC5 inhibitor, providing a novel therapeutic strategy for BC treatment in combination with bortezomib [178]. Additionally, the combination of HDAC inhibitor (Vorinostat) and endocrine therapy (Tamoxifen) showed significant reversal of hormone resistance in ER- positive advanced metastatic BC patients [182].

Potential epi-drug molecules listed in Table 2 have shown promising anticancer effects against breast carcinoma. Promising phase I clinical data have robustly demonstrated that the combination of epigenetic therapies of DNMT and HDAC inhibitors (5-fluoro-2'-deoxycytidine and tetrahydropyridine) was well tolerated. It also reported that this combination has the potential to overcome chemotherapy resistance and

partial response of 16 months in a BC patient [183]. Consequently, a phase II clinical study was conducted to assess response to this combination in patients with advanced BC. Efficacy results of the DNMT and HDAC inhibitors combination suggest that further testing of these drugs is unwarranted in BC [184]. On the other hand, Connolly et al., 2017 reported results from phase II clinical trial, investigated the implementing of combined epigenetic therapies, DNMT and HDAC inhibitors (5-azacitidine (Azacitidine) and entinostat). Finding from this study suggests that some women with advanced hormone-resistant BC may benefit from epigenetic therapy and/or reintroduction of endocrine therapy beyond progression [185].

Currently, implementing epigenetic therapies for BC are still in the early stages and have not moved into routine clinical practice. The investigated DNMT and/or HDAC inhibitors (single and/or combined therapies) have shown encouraging results in BC treatment, nevertheless, these drugs are relatively toxic, and their pharmacodynamics remain nonspecific as gene modulators which consider as major challenges. Also, there are additional limitations which restrict the use of these epigenetic alterations as diagnostic, prognostic biomarker and therapeutic agents. These include the conflicted results due to the use of variable methodologies across different studies, the low load of epigenetic substance in the specimens, and the necessity to enhance purification methods of histone and non-coding RNA. Finally, the epigenetic modifications are usually cell specific which may be directly impacted by external factors such as environment and aging. As a result, these modifications could be non-functional. All these variables should be taken into consideration when selecting epigenetic alteration as a possible cancer specific biomarker [165,186,187].

4. Conclusion

Evidently, epigenetic alterations play an important role in the pathogenesis and poor clinical outcomes of BC via various mechanisms. Consequently, several methylated genes and potential epigenetics inhibitors have been studied and proposed as promising diagnostic, prognostic, and therapeutic agents for BC. A number of studies have reported the feasibility of using methylated genes as potential biomarkers for BC. Nevertheless, currently only two DNA methylation-based assays were developed and validated as prognostic/predictive and diagnostic CE-IVD in the EU and USA (the thetascreen® PITX2 RQG and IvyGene respectively).

Apart from this, accumulating evidence suggests that epigenetic

Table 2
List of potential investigational and approved epigenetic drugs for BC therapy.

Drug Category	Drug Name	Approval	Current Indication
DNMT inhibitors	Azacitidine	FDA approved 2004	Myelodysplastic Syndrome
	Decitabine	FDA approved 2006	Myelodysplastic Syndrome
	5-Fluoro-2-deoxycytidine	Under trials	Solid tumors
	Hydralazine	FDA approved 1997	Hypertention
	Abexinostat	Under trial	follicular lymphoma, solid tumors
	Belinostat	FDA Approved 2014	Peripheral T-cell lymphoma
	CUDC-101	Under trial	Solid tumors
	Entinostat	Under trial	Hodgkin Lymphoma, BC, Kidney Cancer
	Ferrocenyl	Pre-clinical studies	Solid & Soft cancers
	Fingolimod	FDA Approved 2010/18	Adult/Paediatric Multiple sclerosis
HDAC inhibitors	N-(2-hydroxyphenyl)-2-propylpentanamide	Pre-clinical studies	Multiple sclerosis
	Panobinostat	FDA Approved 2015	Multiple Myeloma
	Romidepsin	FDA Approved 2009/12	Peripheral/Cutaneous T-cell lymphoma
	Santacruzamate A	Pre-clinical studies	Solid tumors
	Sodium butyrate	Under trial	Solid tumors
	Tetrahydropyridine	Under trial	Solid tumors, Leukemia
	Trichostatin A	Under trial	Hematologic Malignancies
	Valproic acid	FDA Approved 2008	Epilepsy/Migraine/Mania
	Vorinostat	FDA Approved 2006	Cutaneous T-cell lymphoma
	YCW1	Pre-clinical studies	BC & Lung Cancer
HMT inhibitors	EPZ004777	Pre-clinical studies	Mixed lineage leukemia
	UNC0638	Pre-clinical studies	TNBC & Lung Cancer

therapies could potentially work synergistically, when combined together and/or with conventional chemotherapy, in increasing therapeutic effects. Yet, the findings are not satisfactory and their validation and transfer to the clinical setting is still outstanding. Consequently, this emphasizes the need for further investigations to carefully assess the clinical benefits from implementing these markers. Besides, further clinical trials are necessary to precisely assess and validate the effects of epigenetic modifiers molecules in the treatment of BC. This will facilitate the development of novel reliable biomarkers and effective targeted treatments leading to lower incidence and better management of BC.

Author contributions

Writing—Original draft: N.A.S., G.S., A.Q.K., K.S.P.; Figures and tables: N.A.S.; Supervision: S.U. and N.A.S.; Writing—Review and editing: N.A.S., G.S.; A.R., M.K., S.D., M.H., K.J., S.U.; All authors have read and agreed to the published version of the manuscript.

Funding

The study was supported by grant from the Medical Research Center (to SU; Grants MRC#16354/16, IRGC#05-SI-18-307), Hamad Medical Corporation, Doha, State of Qatar.

Transparency document

The [Transparency document](#) associated with this article can be found in the online version.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgment

The authors acknowledge Qatar National Library fund for supporting the publication.

References

- [1] J. Ferlay, M. Colombet, I. Soerjomataram, C. Mathers, D.M. Parkin, M. Piñeros, et al., Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods, *Int. J. Cancer* 144 (8) (2019) 1941–1953, <https://doi.org/10.1002/ijc.31937>.
- [2] N. Li, Y. Deng, L. Zhou, T. Tian, S. Yang, Y. Wu, et al., Global burden of breast cancer and attributable risk factors in 195 countries and territories, from 1990 to 2017: results from the Global Burden of Disease Study 2017, *J. Hematol. Oncol.* 12 (1) (2019) 140, <https://doi.org/10.1186/s13045-019-0828-0>.
- [3] WHO Report on Cancer: Setting Priorities, Investing Wisely and Providing Care for All, World Health Organization, Geneva, 2020. Licence: CC BY-NC-SA 3.0 IGO. <https://www.who.int/publications/i/item/who-report-on-cancer-setting-priorities-investing-wisely-and-providing-care-for-all>. (Accessed August 6th 2020).
- [4] M. Ghoncheh, Z. Pournamdar, H. Salehiniya, Incidence and mortality and epidemiology of breast Cancer in the world, *Asian Pac. J. Cancer Prev.* 17 (S3) (2016) 43–46, <https://doi.org/10.7314/apjcp.2016.17.s3.43>.
- [5] L.A. Torre, F. Islami, R.L. Siegel, E.M. Ward, A. Jemal, Global Cancer in women: burden and trends, *Cancer Epidemiol. Biomarkers Prev.* 26 (4) (2017) 444–457, <https://doi.org/10.1158/1055-9965.epi-16-0858>.
- [6] G. Turashvili, E. Brogi, Tumor heterogeneity in breast Cancer, *Front. Med. (Lausanne)* 4 (2017) 227, <https://doi.org/10.3389/fmed.2017.00227>.
- [7] H.G. Russnes, O.C. Lingjaerde, A.L. Børresen-Dale, C. Caldas, Breast Cancer Molecular stratification: from intrinsic subtypes to integrative clusters, *Am. J. Pathol.* 187 (10) (2017) 2152–2162, <https://doi.org/10.1016/j.ajpath.2017.04.022>.
- [8] C.A. Iacobuzio-Donahue, K. Litchfield, C. Swanton, Intratumor heterogeneity reflects clinical disease course, *Nat Cancer* 1 (1) (2020) 3–6, <https://doi.org/10.1038/s43018-019-0002-1>.
- [9] M.R. Junttila, F.J. de Sauvage, Influence of tumour micro-environment heterogeneity on therapeutic response, *Nature* 501 (7467) (2013) 346–354, <https://doi.org/10.1038/nature12626>.
- [10] L.A. Hazlehurst, T.H. Landowski, W.S. Dalton, Role of the tumor microenvironment in mediating de novo resistance to drugs and physiological mediators of cell death, *Oncogene* 22 (47) (2003) 7396–7402, <https://doi.org/10.1038/sj.onc.1206943>.
- [11] A. Kreso, J.E. Dick, Evolution of the cancer stem cell model, *Cell Stem Cell* 14 (3) (2014) 275–291, <https://doi.org/10.1016/j.stem.2014.02.006>.
- [12] M.R. Atashzari, R. Baharlou, J. Karami, H. Abdollahi, R. Rezaei, F. Pourramezan, et al., Cancer stem cells: a review from origin to therapeutic implications, *J. Cell. Physiol.* 235 (2) (2020) 790–803, <https://doi.org/10.1002/jcp.29044>.
- [13] M.W. Beckmann, D. Niederacher, H.G. Schnürch, B.A. Gusterson, H.G. Bender, Multistep carcinogenesis of breast cancer and tumour heterogeneity, *J. Mol. Med.* 75 (6) (1997) 429–439, <https://doi.org/10.1007/s001090050128>.
- [14] C. Osborne, P. Wilson, D. Tripathy, Oncogenes and tumor suppressor genes in breast cancer: potential diagnostic and therapeutic applications, *Oncologist* 9 (4) (2004) 361–377, <https://doi.org/10.1634/theoncologist.9-4-361>.
- [15] R. Holliday, Epigenetics: an overview, *Dev. Genet.* 15 (6) (1994) 453–457, <https://doi.org/10.1002/dvg.1020150602>.
- [16] M.A. Dawson, T. Kouzarides, Cancer epigenetics: from mechanism to therapy, *Cell* 150 (1) (2012) 12–27, <https://doi.org/10.1016/j.cell.2012.06.013>.
- [17] B. Pasculli, R. Barbano, P. Parrella, Epigenetics of breast cancer: biology and clinical implication in the era of precision medicine, *Semin. Cancer Biol.* 51 (2018) 22–35, <https://doi.org/10.1016/j.semcancer.2018.01.007>.
- [18] K.D. Robertson, DNA methylation and human disease, *Nat. Rev. Genet.* 6 (8) (2005) 597–610, <https://doi.org/10.1038/nrg1655>.
- [19] F. Lyko, The DNA methyltransferase family: a versatile toolkit for epigenetic regulation, *Nat. Rev. Genet.* 19 (2) (2018) 81–92, <https://doi.org/10.1038/nrg.2017.80>.
- [20] K.D. Robertson, DNA methylation, methyltransferases, and cancer, *Oncogene* 20 (24) (2001) 3139–3155, <https://doi.org/10.1038/sj.onc.1204341>.
- [21] Y. Kurihara, Y. Kawamura, Y. Uchijima, T. Amamo, H. Kobayashi, T. Asano, et al., Maintenance of genomic methylation patterns during preimplantation development requires the somatic form of DNA methyltransferase 1, *Dev. Biol.* 313 (1) (2008) 335–346, <https://doi.org/10.1016/j.ydbio.2007.10.033>.
- [22] P.A. Jones, Functions of DNA methylation: islands, start sites, gene bodies and beyond, *Nat. Rev. Genet.* 13 (7) (2012) 484–492, <https://doi.org/10.1038/nrg.2012.030>.
- [23] A.M. Deaton, A. Bird, CpG islands and the regulation of transcription, *Genes Dev.* 25 (10) (2011) 1010–1022, <https://doi.org/10.1101/gad.2037511>.
- [24] A.R. Brothman, G. Swanson, T.M. Maxwell, J. Cui, K.J. Murphy, J. Herrick, et al., Global hypomethylation is common in prostate cancer cells: a quantitative predictor for clinical outcome? *Cancer Genet. Cytogenet.* 156 (1) (2005) 31–36, <https://doi.org/10.1016/j.cancerycyto.2004.04.004>.
- [25] G.C. Hon, R.D. Hawkins, O.L. Caballero, C. Lo, R. Lister, M. Pelizzola, et al., Global DNA hypomethylation coupled to repressive chromatin domain formation and gene silencing in breast cancer, *Genome Res.* 22 (2) (2012) 246–258, <https://doi.org/10.1101/gr.125872.111>.
- [26] S. Sharma, T.K. Kelly, P.A. Jones, Epigenetics in cancer, *Carcinogenesis* 31 (1) (2010) 27–36, <https://doi.org/10.1093/carcin/bgp220>.
- [27] R. Zelic, V. Fiano, C. Grasso, D. Zugna, A. Pettersson, A. Gillio-Tos, et al., Global DNA hypomethylation in prostate cancer development and progression: a systematic review, *Prostate Cancer Prostatic Dis.* 18 (1) (2015) 1–12, <https://doi.org/10.1038/pcan.2014.45>.
- [28] K. Hur, P. Cejas, J. Feliu, J. Moreno-Rubio, E. Burgos, C.R. Boland, et al., Hypomethylation of long interspersed nuclear element-1 (LINE-1) leads to activation of proto-oncogenes in human colorectal cancer metastasis, *Gut* 63 (4) (2014) 635–646, <https://doi.org/10.1136/gutjnl-2012-304219>.
- [29] M.B. Leick, C.J. Shoff, E.C. Wang, J.L. Congress, G.I. Gallicano, Loss of imprinting of IGF2 and the epigenetic progenitor model of cancer, *Am. J. Stem Cells* 1 (1) (2012) 59–74.
- [30] W. Zhang, J. Xu, DNA methyltransferases and their roles in tumorigenesis, *Biomark. Res.* 5 (2017) 1, <https://doi.org/10.1186/s40364-017-0081-z>.
- [31] R.L. Bennett, J.D. Licht, Targeting epigenetics in Cancer, *Annu. Rev. Pharmacol. Toxicol.* 58 (1) (2018) 187–207, <https://doi.org/10.1146/annurev-pharmtox-010716-105106>.
- [32] J.E. Joo, J.G. Dowty, R.L. Milne, E.M. Wong, P.A. Dugué, D. English, et al., Heritable DNA methylation marks associated with susceptibility to breast cancer, *Nat. Commun.* 9 (1) (2018) 867, <https://doi.org/10.1038/s41467-018-03058-6>.
- [33] L.A. Salas, S.N. Lundgren, E.P. Browne, E.C. Punska, D.L. Anderton, M.R. Karagas, et al., Prediagnostic breast milk DNA methylation alterations in women who develop breast cancer, *Hum. Mol. Genet.* 29 (4) (2020) 662–673, <https://doi.org/10.1093/hmg/ddz301>.
- [34] Q. Tang, J. Cheng, X. Cao, H. Surowy, B. Burwinkel, Blood-based DNA methylation as biomarker for breast cancer: a systematic review, *Clin. Epigenetics* 8 (2016) 115, <https://doi.org/10.1186/s13148-016-0282-6>.
- [35] Y. Liu, X. Jin, Y. Li, Y. Ruan, Y. Lu, M. Yang, et al., DNA methylation of claudin-6 promotes breast cancer cell migration and invasion by recruiting MeCP2 and deacetylating H3Ac and H4Ac, *J. Exp. Clin. Cancer Res.* 35 (1) (2016) 120, <https://doi.org/10.1186/s13046-016-0396-x>.
- [36] B. Györfi, G. Bottai, T. Fleischer, G. Munkácsy, J. Budczies, L. Paladini, et al., Aberrant DNA methylation impacts gene expression and prognosis in breast cancer subtypes, *Int. J. Cancer* 138 (1) (2016) 87–97, <https://doi.org/10.1002/ijc.29684>.
- [37] S. Masood, E. El-Gabry, C. Zhang, Z. Wang, DNA methylation of the hTERT gene in breast Cancer revisited: diagnostic and clinical implications, *Lab. Med.* 47 (4) (2016) 293–299, <https://doi.org/10.1093/labmed/lmw043>.
- [38] T. Fleischer, X. Tekpli, A. Mathelier, S. Wang, D. Nebdal, H.P. Dhakal, et al., DNA methylation at enhancers identifies distinct breast cancer lineages, *Nat. Commun.* 8 (1) (2017) 1379, <https://doi.org/10.1038/s41467-017-00510-x>.

- [39] W. Jin, Q.Z. Li, Y.C. Zuo, Y.N. Cao, L.Q. Zhang, R. Hou, et al., Relationship between DNA methylation in key region and the differential expressions of genes in human breast tumor tissue, *DNA Cell Biol.* 38 (1) (2019) 49–62, <https://doi.org/10.1089/dna.2018.4276>.
- [40] S. Ambatipudi, S. Horvath, F. Perrier, C. Cuenin, H. Hernandez-Vargas, F. Le Calvez-Kelm, et al., DNA methylome analysis identifies accelerated epigenetic ageing associated with postmenopausal breast cancer susceptibility, *Eur. J. Cancer* 75 (2017) 299–307, <https://doi.org/10.1016/j.ejca.2017.01.014>.
- [41] B. Griess, D. Klinkebiel, A. Kueh, M. Desler, K. Cowan, M. Fitzgerald, et al., Association of SOD3 promoter DNA methylation with its down-regulation in breast carcinomas, *Epigenetics* (2020) 1–11, <https://doi.org/10.1080/15592294.2020.1777666>.
- [42] C.R. Good, S. Panjarian, A.D. Kelly, J. Madzo, B. Patel, J. Jelinek, et al., TET1-mediated hypomethylation activates oncogenic signaling in triple-negative breast Cancer, *Cancer Res.* 78 (15) (2018) 4126–4137, <https://doi.org/10.1158/0008-5472.CAN-17-2082>.
- [43] J. Gómez-Miragaya, S. Morán, M.E. Calleja-Cervantes, A. Collado-Sole, L. Paré, A. Gómez, et al., The altered transcriptome and DNA methylation profiles of docetaxel resistance in breast cancer pdx models, *Mol. Cancer Res.* 17 (10) (2019) 2063–2076, <https://doi.org/10.1158/1541-7786.mcr-19-0040>.
- [44] S. Gkoutela, F. Castro-Giner, B.M. Szczerba, M. Vetter, J. Landin, R. Scherrer, et al., Circulating tumor cell clustering shapes DNA methylation to enable metastasis seeding, *Cell* 176 (1–2) (2019) 98–112, <https://doi.org/10.1016/j.cell.2018.11.046>, e14.
- [45] M. Shah, R. Cardenas, B. Wang, J. Persson, N.P. Mongan, A. Grabowska, et al., HOXC8 regulates self-renewal, differentiation and transformation of breast cancer stem cells, *Mol. Cancer* 16 (1) (2017) 38, <https://doi.org/10.1186/s12943-017-0605-z>.
- [46] P.M. Wörner, D.J. Schächtele, Z. Barabadi, S. Srivastav, B. Chandrasekar, R. Izadpanah, et al., Breast tumor microenvironment can transform naïve mesenchymal stem cells into tumor-forming cells in nude mice, *Stem Cells Dev.* 28 (5) (2019) 341–352, <https://doi.org/10.1089/scd.2018.0110>.
- [47] W. Shi, T. Tang, X. Li, S. Deng, R. Li, Y. Wang, et al., Methylation-mediated silencing of miR-133a-3p promotes breast cancer cell migration and stemness via miR-133a-3p/MAML1/DNMT3A positive feedback loop, *J. Exp. Clin. Cancer Res.* 38 (1) (2019) 429, <https://doi.org/10.1186/s13046-019-1400-z>.
- [48] L. Zhang, D.L. Yan, F. Yang, D.D. Wang, X. Chen, J.Z. Wu, et al., DNA methylation mediated silencing of microRNA-874 is a promising diagnosis and prognostic marker in breast cancer, *Oncotarget* 8 (28) (2017) 45496–45505, <https://doi.org/10.18632/oncotarget.17569>.
- [49] I.V. Pronina, V.I. Loginov, A.M. Burdennny, M.V. Fridman, V.N. Senchenko, T. P. Kazubskaya, et al., DNA methylation contributes to deregulation of 12 cancer-associated microRNAs and breast cancer progression, *Gene* 604 (2017) 1–8, <https://doi.org/10.1016/j.gene.2016.12.018>.
- [50] J. Achinger-Kawecka, F. Valdes-Mora, P.-L. Luu, K.A. Giles, C.E. Caldon, W. Qu, et al., Epigenetic reprogramming at estrogen-receptor binding sites alters 3D chromatin landscape in endocrine-resistant breast cancer, *Nat. Commun.* 11 (1) (2020) 320, <https://doi.org/10.1038/s41467-019-14098-x>.
- [51] C.G. Kleer, Q. Cao, S. Varambally, R. Shen, I. Ota, S.A. Tomlins, et al., EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells, *Proc Natl Acad Sci U S A* 100 (20) (2003) 11606–11611, <https://doi.org/10.1073/pnas.1933744100>.
- [52] L. Ding, C. Erdmann, A.M. Chinnaiyan, S.D. Merajver, C.G. Kleer, Identification of EZH2 as a molecular marker for a precancerous state in morphologically normal breast tissues, *Cancer Res.* 66 (8) (2006) 4095–4099, <https://doi.org/10.1158/0008-5472.CAN-05-4300>.
- [53] Y. Wu, Z. Zhang, M.E. Cenciari, C.J. Proietti, M. Amasino, T. Hong, et al., Tamoxifen resistance in breast Cancer is regulated by the EZH2-ERα-GREB1 transcriptional Axis, *Cancer Res.* 78 (3) (2018) 671–684, <https://doi.org/10.1158/0008-5472.can-17-1327>.
- [54] S. Palomeras, Á. Diaz-Lagares, G. Viñas, F. Setien, H.J. Ferreira, G. Oliveras, et al., Epigenetic silencing of TGFBI confers resistance to trastuzumab in human breast cancer, *Breast Cancer Res.* 21 (1) (2019) 79, <https://doi.org/10.1186/s13058-019-1160-x>.
- [55] L. Ye, C. Lin, X. Wang, Q. Li, Y. Li, M. Wang, et al., Epigenetic silencing of SALL2 confers tamoxifen resistance in breast cancer, *EMBO Mol. Med.* 11 (12) (2019), e10638, <https://doi.org/10.15252/emmm.201910638>.
- [56] F. Liu, F. Ma, Y. Wang, L. Hao, H. Zeng, C. Jia, et al., PKM2 methylation by CARM1 activates aerobic glycolysis to promote tumorigenesis, *Nat. Cell Biol.* 19 (11) (2017) 1358–1370, <https://doi.org/10.1038/ncb3630>.
- [57] M. Esteller, Cancer epigenomics: DNA methylomes and histone-modification maps, *Nat. Rev. Genet.* 8 (4) (2007) 286–298, <https://doi.org/10.1038/nrg2005>.
- [58] E.V. Benevolenskaya, A.B. Islam, H. Ahsan, M.G. Kibriya, F. Jasmine, B. Wolff, et al., DNA methylation and hormone receptor status in breast cancer, *Clin. Epigenetics* 8 (2016) 17, <https://doi.org/10.1186/s13148-016-0184-7>.
- [59] M. Boettcher, F. Kischkel, J.D. Hoheisel, High-definition DNA methylation profiles from breast and ovarian carcinoma cell lines with differing doxorubicin resistance, *PLoS One* 5 (6) (2010), e11002, <https://doi.org/10.1371/journal.pone.0011002>.
- [60] V.F. Chekhun, N.Y. Lukyanova, O. Kovalchuk, V.P. Tryndyak, I.P. Pogribny, Epigenetic profiling of multidrug-resistant human MCF-7 breast adenocarcinoma cells reveals novel hyper- and hypomethylated targets, *Mol. Cancer Ther.* 6 (3) (2007) 1089–1098, <https://doi.org/10.1158/1535-7163.MCT-06-0663>.
- [61] T.G. Oh, S.M. Wang, G.E. Muscat, Therapeutic implications of epigenetic signaling in breast Cancer, *Endocrinology* 158 (3) (2017) 431–447, <https://doi.org/10.1210/en.2016-1716>.
- [62] B. Györfy, A. Lanczky, A.C. Eklund, C. Denkert, J. Budczies, Q. Li, et al., An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients, *Breast Cancer Res. Treat.* 123 (3) (2010) 725–731, <https://doi.org/10.1007/s10549-009-0674-9>.
- [63] J.Y. Chen, C.F. Li, P.Y. Chu, Y.S. Lai, C.H. Chen, S.S. Jiang, et al., Lysine demethylase 2A promotes stemness and angiogenesis of breast cancer by upregulating Jagged1, *Oncotarget* 7 (19) (2016) 27689–27710, <https://doi.org/10.18632/oncotarget.8381>.
- [64] J.M. Pang, A. Dobrovic, S.B. Fox, DNA methylation in ductal carcinoma in situ of the breast, *Breast Cancer Res.* 15 (3) (2013) 206, <https://doi.org/10.1186/bcr3420>.
- [65] B. Downs, S.M. Wang, Epigenetic changes in BRCA1-mutated familial breast cancer, *Cancer Genet.* 208 (5) (2015) 237–240, <https://doi.org/10.1016/j.cancergen.2015.02.001>.
- [66] L. Ponnusamy, P.K.S. Mahalingaiah, Y.W. Chang, K.P. Singh, Reversal of epigenetic aberrations associated with the acquisition of doxorubicin resistance restores drug sensitivity in breast cancer cells, *Eur. J. Pharm. Sci.* 123 (2018) 56–69, <https://doi.org/10.1016/j.ejps.2018.07.028>.
- [67] H.G. Chang, S.J. Kim, K.W. Chung, D.Y. Noh, Y. Kwon, E.S. Lee, et al., Tamoxifen-resistant breast cancers show less frequent methylation of the estrogen receptor beta but not the estrogen receptor alpha gene, *J. Mol. Med.* 83 (2) (2005) 132–139, <https://doi.org/10.1007/s00109-004-0596-2>.
- [68] P. Mathot, M. Grandin, G. Devailly, F. Souaze, V. Cahais, S. Moran, et al., DNA methylation signal has a major role in the response of human breast cancer cells to the microenvironment, *Oncogenesis* 6 (10) (2017), e390, <https://doi.org/10.1038/oncsis.2017.88>.
- [69] E. Toska, H.U. Osmanbeyoglu, P. Castel, C. Chan, R.C. Hendrickson, M. Elkabets, et al., PI3K pathway regulates ER-dependent transcription in breast cancer through the epigenetic regulator KMT2D, *Science* 355 (6331) (2017) 1324–1330, <https://doi.org/10.1126/science.aah6893>.
- [70] M. Widschwendter, K.D. Siegmund, H.M. Müller, H. Fiegl, C. Marth, E. Müller-Holzner, et al., Association of breast cancer DNA methylation profiles with hormone receptor status and response to tamoxifen, *Cancer Res.* 64 (11) (2004) 3807–3813, <https://doi.org/10.1158/0008-5472.CAN-03-3852>.
- [71] H. Fiegl, S. Millinger, G. Goebel, E. Müller-Holzner, C. Marth, P.W. Laird, et al., Breast cancer DNA methylation profiles in cancer cells and tumor stroma: association with HER-2/neu status in primary breast cancer, *Cancer Res.* 66 (1) (2006) 29–33, <https://doi.org/10.1158/0008-5472.CAN-05-2508>.
- [72] M. Hu, J. Yao, L. Cai, K.E. Bachman, F. van den Brûle, V. Velculescu, et al., Distinct epigenetic changes in the stromal cells of breast cancers, *Nat. Genet.* 37 (8) (2005) 899–905, <https://doi.org/10.1038/ng1596>.
- [73] L. He, H. Li, A. Wu, Y. Peng, G. Shu, G. Yin, Functions of N6-methyladenosine and its role in cancer, *Mol. Cancer* 18 (1) (2019) 176, <https://doi.org/10.1186/s12943-019-1109-9>.
- [74] H. Wang, B. Xu, J. Shi, N6-methyladenosine METTL3 promotes the breast cancer progression via targeting Bcl-2, *Gene* 722 (2020), 144076, <https://doi.org/10.1016/j.gene.2019.144076>.
- [75] Y. Niu, Z. Lin, A. Wan, H. Chen, H. Liang, L. Sun, et al., RNA N6-methyladenosine demethylase FTO promotes breast tumor progression through inhibiting BNIP3, *Mol. Cancer* 18 (1) (2019) 46, <https://doi.org/10.1186/s12943-019-1004-x>.
- [76] C. Zhang, D. Samanta, H. Lu, J.W. Bullen, H. Zhang, I. Chen, et al., Hypoxia induces the breast cancer stem cell phenotype by HIF-dependent and ALKBH5-mediated m⁶A-demethylation of NANOG mRNA, *Proc. Natl. Acad. Sci. U.S.A.* 113 (14) (2016) E2047–E2056, <https://doi.org/10.1073/pnas.1602883113>.
- [77] C. Zhang, W.I. Zhi, H. Lu, D. Samanta, I. Chen, E. Gabrielson, et al., Hypoxia-inducible factors regulate pluripotency factor expression by ZNF217- and ALKBH5-mediated modulation of RNA methylation in breast cancer cells, *Oncotarget* 7 (40) (2016) 64527–64542, <https://doi.org/10.18632/oncotarget.11743>.
- [78] L. Wu, D. Wu, J. Ning, W. Liu, D. Zhang, Changes of N6-methyladenosine modulators promote breast cancer progression, *BMC Cancer* 19 (1) (2019) 326, <https://doi.org/10.1186/s12885-019-5538-z>.
- [79] L. Liu, X. Liu, Z. Dong, J. Li, Y. Yu, X. Chen, et al., N6-methyladenosine-related genomic targets are altered in breast Cancer tissue and associated with poor survival, *J. Cancer* 10 (22) (2019) 5447–5459, <https://doi.org/10.7150/jca.35053>.
- [80] Q.Y. Zhao, P.J. Lei, X. Zhang, J.Y. Zheng, H.Y. Wang, J. Zhao, et al., Global histone modification profiling reveals the epigenomic dynamics during malignant transformation in a four-stage breast cancer model, *Clin. Epigenetics* 8 (2016) 34, <https://doi.org/10.1186/s13148-016-0201-x>.
- [81] O. Müller, N. Kepper, R. Schöpf, R. Ettig, K. Rippe, G. Wedemann, Changing chromatin fiber conformation by nucleosome repositioning, *Biophys. J.* 107 (9) (2014) 2141–2150, <https://doi.org/10.1016/j.bpj.2014.09.026>.
- [82] Z. Wang, D.E. Schones, K. Zhao, Characterization of human epigenomes, *Curr. Opin. Genet. Dev.* 19 (2) (2009) 127–134, <https://doi.org/10.1016/j.gde.2009.02.001>.
- [83] S.G. Swygert, C.L. Peterson, Chromatin dynamics: interplay between remodeling enzymes and histone modifications, *Biochim. Biophys. Acta* 1839 (8) (2014) 728–736, <https://doi.org/10.1016/j.bbagr.2014.02.013>.
- [84] R.Y. Tweedie-Cullen, A.M. Brunner, J. Grossmann, S. Mohanna, D. Sichau, P. Nanni, et al., Identification of combinatorial patterns of post-translational modifications on individual histones in the mouse brain, *PLoS One* 7 (5) (2012), e36980, <https://doi.org/10.1371/journal.pone.0036980>.
- [85] J. Dieker, S. Muller, Epigenetic histone code and autoimmunity, *Clin. Rev. Allergy Immunol.* 39 (1) (2010) 78–84, <https://doi.org/10.1007/s12016-009-8173-7>.

- [86] P. Guo, W. Chen, H. Li, M. Li, L. Li, The histone acetylation modifications of breast Cancer and their therapeutic implications, *Pathol. Oncol. Res.* 24 (4) (2018) 807–813, <https://doi.org/10.1007/s12253-018-0433-5>.
- [87] Y. Xi, J. Shi, W. Li, K. Tanaka, K.L. Allton, D. Richardson, et al., Histone modification profiling in breast cancer cell lines highlights commonalities and differences among subtypes, *BMC Genomics* 19 (1) (2018) 150, <https://doi.org/10.1186/s12864-018-4533-0>.
- [88] S.E. Elsheikh, A.R. Green, E.A. Rakha, D.G. Powe, R.A. Ahmed, H.M. Collins, et al., Global histone modifications in breast cancer correlate with tumor phenotypes, prognostic factors, and patient outcome, *Cancer Res.* 69 (9) (2009) 3802–3809, <https://doi.org/10.1158/0008-5472.CAN-08-3907>.
- [89] C. He, J.M. Danes, P.C. Hart, Y. Zhu, Y. Huang, A.L. de Abreu, et al., SOD2 acetylation on lysine 68 promotes stem cell reprogramming in breast cancer, *Proc. Natl. Acad. Sci. U.S.A.* 116 (47) (2019) 23534–23541, <https://doi.org/10.1073/pnas.1902308116>.
- [90] C. Wang, Z. Zhou, C.S. Subramanyam, Q. Cao, Z.S.L. Heng, W. Liu, et al., SRPK1 acetylation modulates alternative splicing to regulate cisplatin resistance in breast cancer cells, *Commun Biol* 3 (1) (2020) 268, <https://doi.org/10.1038/s42003-020-0983-4>.
- [91] B. Chatterjee, K. Ghosh, S.R. Kanade, Resveratrol modulates epigenetic regulators of promoter histone methylation and acetylation that restores BRCA1, p53, p21 (CIP1) in human breast cancer cell lines, *Biofactors* 45 (5) (2019) 818–829, <https://doi.org/10.1002/biof.1544>.
- [92] C. Caslini, S. Hong, Y.J. Ban, X.S. Chen, T.A. Ince, HDAC7 regulates histone 3 lysine 27 acetylation and transcriptional activity at super-enhancer-associated genes in breast cancer stem cells, *Oncogene* 38 (39) (2019) 6599–6614, <https://doi.org/10.1038/s41388-019-0897-0>.
- [93] Y. Li, E. Seto, HDACs and HDAC inhibitors in Cancer development and therapy, *Cold Spring Harb. Perspect. Med.* 6 (10) (2016), <https://doi.org/10.1101/cshperspect.a026831>.
- [94] D. You, J.R. Richardson, L.M. Aleksunes, Epigenetic regulation of multidrug resistance protein 1 and breast Cancer resistance protein transporters by histone deacetylase inhibition, *Drug Metab. Dispos.* 48 (6) (2020) 459–480, <https://doi.org/10.1124/dmd.119.089953>.
- [95] A. Sharda, M. Rashid, S.G. Shah, A.K. Sharma, S.R. Singh, P. Gera, et al., Elevated HDAC activity and altered histone phospho-acetylation confer acquired radio-resistant phenotype to breast cancer cells, *Clin. Epigenetics* 12 (1) (2020) 4, <https://doi.org/10.1186/s13148-019-0800-4>.
- [96] J. Seo, S.K. Min, H.R. Park, D.H. Kim, M.J. Kwon, L.S. Kim, et al., Expression of histone deacetylases HDAC1, HDAC2, HDAC3, and HDAC6 in invasive ductal carcinomas of the breast, *J. Breast Cancer* 17 (4) (2014) 323–331, <https://doi.org/10.4048/jbc.2014.17.4.323>.
- [97] R.S. DeVaux, J.I. Herschkowitz, Beyond DNA: the role of epigenetics in the premalignant progression of breast Cancer, *J. Mammary Gland Biol. Neoplasia* 23 (4) (2018) 223–235, <https://doi.org/10.1007/s10911-018-9414-2>.
- [98] Z. Zhang, H. Yamashita, T. Toyama, H. Sugiyama, Y. Ando, K. Mita, et al., Quantitation of HDAC1 mRNA expression in invasive carcinoma of the breast*, *Breast Cancer Res. Treat.* 94 (1) (2005) 11–16, <https://doi.org/10.1007/s10549-005-6001-1>.
- [99] B.M. Müller, L. Jana, A. Kasajima, A. Lehmann, J. Prinzler, J. Budczies, et al., Differential expression of histone deacetylases HDAC1, 2 and 3 in human breast cancer—overexpression of HDAC2 and HDAC3 is associated with clinicopathological indicators of disease progression, *BMC Cancer* 13 (2013) 215, <https://doi.org/10.1186/1471-2407-13-215>.
- [100] M. Lapierre, A. Linares, M. Dalvai, C. Duraffourd, S. Bonnet, A. Boulahtouf, et al., Histone deacetylase 9 regulates breast cancer cell proliferation and the response to histone deacetylase inhibitors, *Oncotarget* 7 (15) (2016) 19693–19708, <https://doi.org/10.18632/oncotarget.7564>.
- [101] C. Gomez-Moreno, New roles of flavoproteins in molecular cell biology, *FEBS J.* 276 (16) (2009) 4289, [https://doi.org/EJB7140 \[pii\] 10.1111/j.1742-4658.2009.07140.x](https://doi.org/EJB7140 [pii] 10.1111/j.1742-4658.2009.07140.x).
- [102] S. Lim, A. Janzer, A. Becker, A. Zimmer, R. Schüle, R. Buettner, et al., Lysine-specific demethylase 1 (LSD1) is highly expressed in ER-negative breast cancers and a biomarker predicting aggressive biology, *Carcinogenesis* 31 (3) (2010) 512–520, <https://doi.org/10.1093/carcin/bgp324>.
- [103] N. Serce, A. Gnatzy, S. Steiner, H. Lorenzen, J. Kirfel, R. Buettner, Elevated expression of LSD1 (Lysine-specific demethylase 1) during tumour progression from pre-invasive to invasive ductal carcinoma of the breast, *BMC Clin. Pathol.* 12 (2012) 13, <https://doi.org/10.1186/1472-6890-12-13>.
- [104] S.W. Tyan, C.H. Hsu, K.L. Peng, C.C. Chen, W.H. Kuo, E.Y. Lee, et al., Breast cancer cells induce stromal fibroblasts to secrete ADAMTS1 for cancer invasion through an epigenetic change, *PLoS One* 7 (4) (2012), e35128, <https://doi.org/10.1371/journal.pone.0035128>.
- [105] M. Saxena, R.K.R. Kalathur, N. Rubinstein, A. Vettiger, N. Sugiyama, M. Neutzner, et al., A Pygopus 2-histone interaction is critical for cancer cell de-differentiation and progression in malignant breast cancer, *Cancer Res.* (2020), <https://doi.org/10.1158/0008-5472.CAN-19-2910>.
- [106] J. Verigos, P. Karakaidos, D. Kordias, A. Papoudou-Bai, Z. Evangelou, H. V. Harisis, et al., The Histone Demethylase LSD1/KDM1A Mediates Chemoresistance in Breast Cancer via Regulation of a Stem Cell Program, *Cancers* (Basel) 11 (10) (2019), <https://doi.org/10.3390/cancers11101585>.
- [107] Z. Meng, Y. Liu, J. Wang, H. Fan, H. Fang, S. Li, et al., Histone demethylase KDM7A is required for stem cell maintenance and apoptosis inhibition in breast cancer, *J. Cell. Physiol.* 235 (2) (2020) 932–943, <https://doi.org/10.1002/jcp.29008>.
- [108] A. Nihan Kilinc, N. Sugiyama, R.K. Reddy Kalathur, H. Antoniadis, H. Birogul, D. Ishay-Ronen, et al., Histone deacetylases, Mbd3/NuRD, and Tet2 hydroxylase are crucial regulators of epithelial-mesenchymal plasticity and tumor metastasis, *Oncogene* 39 (7) (2020) 1498–1513, <https://doi.org/10.1038/s41388-019-1081-2>.
- [109] Y. Wang, J. Zeng, W. Wu, S. Xie, H. Yu, G. Li, et al., Nicotinamide N-methyltransferase enhances chemoresistance in breast cancer through SIRT1 protein stabilization, *Breast Cancer Res.* 21 (1) (2019) 64, <https://doi.org/10.1186/s13058-019-1150-z>.
- [110] K.W. Nettles, G. Gil, J. Nowak, R. Métié, V.B. Sharma, G.L. Greene, CBP is a dosage-dependent regulator of nuclear factor-kappaB suppression by the estrogen receptor, *Mol. Endocrinol.* 22 (2) (2008) 263–272, <https://doi.org/10.1210/me.2007-0324>.
- [111] G.M. Pao, R. Janknecht, H. Ruffner, T. Hunter, I.M. Verma, CBP/p300 interact with and function as transcriptional coactivators of BRCA1, *Proc Natl Acad Sci U S A* 97 (3) (2000) 1020–1025, <https://doi.org/10.1073/pnas.97.3.1020>.
- [112] J. Chen, Z. Miao, B. Xue, Y. Shan, G. Weng, B. Shen, Long non-coding RNAs in urologic malignancies: functional roles and clinical translation, *J. Cancer* 7 (13) (2016) 1842–1855, <https://doi.org/10.7150/jca.15876>.
- [113] P. Barata, A.K. Sood, D.S. Hong, RNA-targeted therapeutics in cancer clinical trials: current status and future directions, *Cancer Treat. Rev.* 50 (2016) 35–47, <https://doi.org/10.1016/j.ctrv.2016.08.004>.
- [114] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, *Cell* 116 (2) (2004) 281–297, [https://doi.org/10.1016/s0092-8674\(04\)00045-5](https://doi.org/10.1016/s0092-8674(04)00045-5).
- [115] J.A. Martens, L. Laprade, F. Winston, Intergenic transcription is required to repress the *Saccharomyces cerevisiae* SER3 gene, *Nature* 429 (6991) (2004) 571–574, <https://doi.org/10.1038/nature02538>.
- [116] I. Martianov, A. Ramadass, A. Serra Barros, N. Chow, A. Akoulitchev, Repression of the human dihydrofolate reductase gene by a non-coding interfering transcript, *Nature* 445 (7128) (2007) 666–670, <https://doi.org/10.1038/nature05519>.
- [117] J. Chaumel, P. Le Baccon, A. Wutz, E. Heard, A novel role for Xist RNA in the formation of a repressive nuclear compartment into which genes are recruited when silenced, *Genes Dev.* 20 (16) (2006) 2223–2237, <https://doi.org/10.1101/gad.380906>.
- [118] J. Feng, C. Bi, B.S. Clark, R. Mady, P. Shah, J.D. Kohtz, The Evt-2 noncoding RNA is transcribed from the Dlx-5/6 ultraconserved region and functions as a Dlx-2 transcriptional coactivator, *Genes Dev.* 20 (11) (2006) 1470–1484, <https://doi.org/10.1101/gad.1416106>.
- [119] A.T. Willingham, A.P. Orth, S. Batalov, E.C. Peters, B.G. Wen, P. Aza-Blanc, et al., A strategy for probing the function of noncoding RNAs finds a repressor of NFAT, *Science* 309 (5740) (2005) 1570–1573, <https://doi.org/10.1126/science.1115901>.
- [120] V.T. Nguyen, T. Kiss, A.A. Michels, O. Bensaude, 7SK small nuclear RNA binds to and inhibits the activity of CDK9/cyclin T complexes, *Nature* 414 (6861) (2001) 322–325, <https://doi.org/10.1038/35104581>.
- [121] I. Penna, A. Gigoni, D. Costa, S. Vella, D. Russo, A. Poggi, et al., The inhibition of 45A ncRNA expression reduces tumor formation, affecting tumor nodules compactness and metastatic potential in neuroblastoma cells, *Oncotarget* 8 (5) (2017) 8189–8205, <https://doi.org/10.18632/oncotarget.14138>.
- [122] R. Tripathi, I. Aier, P. Chakraborty, P.K. Varadwaj, Unravelling the role of long non-coding RNA - LINC01087 in breast cancer, *Noncoding RNA Res.* 5 (1) (2020) 1–10, <https://doi.org/10.1016/j.ncrna.2019.12.002>.
- [123] R.A. Youness, M.Z. Gad, Long non-coding RNAs: functional regulatory players in breast cancer, *Noncoding RNA Res.* 4 (1) (2019) 36–44, <https://doi.org/10.1016/j.ncrna.2019.01.003>.
- [124] O. Zaheed, J. Samson, K. Dean, A bioinformatics approach to identify novel long, non-coding RNAs in breast cancer cell lines from an existing RNA-sequencing dataset, *Noncoding RNA Res.* 5 (2) (2020) 48–59, <https://doi.org/10.1016/j.ncrna.2020.02.004>.
- [125] K.K. Thammiah, S. Jayaram, Role of let-7 family microRNA in breast cancer, *Noncoding RNA Res.* 1 (1) (2016) 77–82, <https://doi.org/10.1016/j.ncrna.2016.10.003>.
- [126] Z. Jiang, P. Cheng, B. Luo, J. Huang, Construction and analysis of a long non-coding RNA-Associated competing endogenous RNA network identified potential prognostic biomarkers in luminal breast Cancer, *Oncotarget* 13 (2020) 4271–4282, <https://doi.org/10.2147/ott.s240973>.
- [127] Z. Pan, J. Ding, Z. Yang, H. Li, H. Ding, Q. Chen, LncRNA FLVCR1-AS1 promotes proliferation, migration and activates Wnt/β-catenin pathway through miR-381-3p/CTNBN1 axis in breast cancer, *Cancer Cell Int.* 20 (2020) 214, <https://doi.org/10.1186/s12935-020-01247-2>.
- [128] K.C. Johnson, D.C. Koestler, T. Fleischer, P. Chen, E.G. Jensen, J.D. Marotti, et al., DNA methylation in ductal carcinoma in situ related with future development of invasive breast cancer, *Clin. Epigenetics* 7 (1) (2015) 75, <https://doi.org/10.1186/s13148-015-0094-0>.
- [129] R.A. Gupta, N. Shah, K.C. Wang, J. Kim, H.M. Horlings, D.J. Wong, et al., Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis, *Nature* 464 (7291) (2010) 1071–1076, <https://doi.org/10.1038/nature08975>.
- [130] S.J. Shi, L.J. Wang, B. Yu, Y.H. Li, Y. Jin, X.Z. Bai, LncRNA-ATB promotes trastuzumab resistance and invasion-metastasis cascade in breast cancer, *Oncotarget* 6 (13) (2015) 11652–11663, <https://doi.org/10.18632/oncotarget.3457>.
- [131] X. Si, R. Zang, E. Zhang, Y. Liu, X. Shi, L. Shao, et al., LncRNA H19 confers chemoresistance in ERα-positive breast cancer through epigenetic silencing of the pro-apoptotic gene BIK, *Oncotarget* 7 (49) (2016) 81452–81462, <https://doi.org/10.18632/oncotarget.13263>.

- [132] K.J. Zhang, X.L. Tan, L. Guo, The long non-coding RNA DANCER regulates the inflammatory phenotype of breast cancer cells and promotes breast cancer progression via EZH2-dependent suppression of SOCS3 transcription, *Mol. Oncol.* 14 (2) (2020) 309–328, <https://doi.org/10.1002/1878-0261.12622>.
- [133] B. Jiang, Y. Li, X. Qu, H. Zhu, Y. Tan, Q. Fan, et al., Long noncoding RNA cancer susceptibility candidate 9 promotes doxorubicin-resistant breast cancer by binding to enhancer of zeste homolog 2, *Int. J. Mol. Med.* 42 (5) (2018) 2801–2810, <https://doi.org/10.3892/ijmm.2018.3812>.
- [134] X. Ji, Y. Lu, H. Tian, X. Meng, M. Wei, W.C. Cho, Chemoresistance mechanisms of breast cancer and their countermeasures, *Biomed. Pharmacother.* 114 (2019), 108800, <https://doi.org/10.1016/j.biopharm.2019.108800>.
- [135] M.M. Haque, K.V. Desai, Pathways to endocrine therapy resistance in breast Cancer, *Front. Endocrinol. (Lausanne)* 10 (2019) 573, <https://doi.org/10.3389/fendo.2019.00573>.
- [136] P. Muluhngwi, C.M. Klinge, Roles for miRNAs in endocrine resistance in breast cancer, *Endocr. Relat. Cancer* 22 (5) (2015) R279–R300, <https://doi.org/10.1530/erc-15-0355>.
- [137] Q.X. Luan, B.G. Zhang, X.J. Li, M.Y. Guo, MiR-129-5p is downregulated in breast cancer cells partly due to promoter H3K27m3 modification and regulates epithelial-mesenchymal transition and multi-drug resistance, *Eur. Rev. Med. Pharmacol. Sci.* 20 (20) (2016) 4257–4265.
- [138] D.R. Cochrane, N.S. Spoelstra, E.N. Howe, S.K. Nordeen, J.K. Richer, MicroRNA-200c mitigates invasiveness and restores sensitivity to microtubule-targeting chemotherapeutic agents, *Mol. Cancer Ther.* 8 (5) (2009) 1055–1066, <https://doi.org/10.1158/1535-7163.mct-08-1046>.
- [139] M. Zhou, Z. Liu, Y. Zhao, Y. Ding, H. Liu, Y. Xi, et al., MicroRNA-125b confers the resistance of breast cancer cells to paclitaxel through suppression of pro-apoptotic Bcl-2 antagonist killer 1 (Bak1) expression, *J. Biol. Chem.* 285 (28) (2010) 21496–21507, <https://doi.org/10.1074/jbc.M109.083337>.
- [140] X. He, X. Xiao, L. Dong, N. Wan, Z. Zhou, H. Deng, et al., MiR-218 regulates cisplatin chemosensitivity in breast cancer by targeting BRCA1, *Tumour Biol.* 36 (3) (2015) 2065–2075, <https://doi.org/10.1007/s13277-014-2814-z>.
- [141] S.H. Shah, P. Miller, M. Garcia-Contreras, Z. Ao, L. Machlin, E. Issa, et al., Hierarchical paracrine interaction of breast cancer associated fibroblasts with cancer cells via hMAPK-microRNAs to drive ER-negative breast cancer phenotype, *Cancer Biol. Ther.* 16 (11) (2015) 1671–1681, <https://doi.org/10.1080/15384047.2015.1071742>.
- [142] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *CA Cancer J. Clin.* 68 (6) (2018) 394–424, <https://doi.org/10.3322/caac.21492>.
- [143] P. Zubor, P. Kubatka, K. Kajo, Z. Dankova, H. Polacek, T. Bielik, et al., Why the gold standard approach by mammography demands extension by Multiomics? Application of liquid biopsy miRNA profiles to breast cancer disease management, *Int. J. Mol. Sci.* 20 (12) (2019), <https://doi.org/10.3390/ijms20122878>.
- [144] T.-X. Xiang, Y. Yuan, L.-L. Li, Z.-H. Wang, L.-Y. Dan, Y. Chen, et al., Aberrant promoter CpG methylation and its translational applications in breast cancer, *Chin. J. Cancer* 32 (1) (2013) 12–20, <https://doi.org/10.5732/cjc.011.10344>.
- [145] M.-C. Pouliot, Y. Labrie, C. Diorio, F. Durocher, The role of methylation in breast cancer susceptibility and treatment, *Anticancer Res.* 35 (9) (2015) 4569–4574.
- [146] P. Costa-Pinheiro, D. Montezuma, R. Henrique, C. Jerónimo, Diagnostic and prognostic epigenetic biomarkers in cancer, *Epigenomics* 7 (6) (2015) 1003–1015, <https://doi.org/10.2217/epi.15.56>.
- [147] C. Basse, M. Arock, The increasing roles of epigenetics in breast cancer: implications for pathogenicity, biomarkers, prevention and treatment, *Int. J. Cancer* 137 (12) (2015) 2785–2794, <https://doi.org/10.1002/ijc.29347>.
- [148] M.B. Terry, J.A. McDonald, H.C. Wu, S. Eng, R.M. Santella, Epigenetic biomarkers of breast Cancer risk: across the breast cancer prevention continuum, *Adv. Exp. Med. Biol.* 882 (2016) 33–68, https://doi.org/10.1007/978-3-319-22909-6_2.
- [149] P. Manasa, Epigenetics—role as biomarker in cancer diagnosis, *J. Oncol. Res. Treat* 2 (2017) 114.
- [150] G. Egger, Epigenetic biomarkers in cancer, *ESMO Open* 3 (5) (2018), e000416, <https://doi.org/10.1136/esmoopen-2018-000416>.
- [151] S. Salta, S. P. Nunes, M. Fontes-Sousa, P. Lopes, M. Freitas, M. Caldas, et al., A DNA methylation-based test for breast cancer detection in circulating cell-free DNA, *J. Clin. Med.* 7 (11) (2018), <https://doi.org/10.3390/jcm7110420>.
- [152] Z. Xu, D.P. Sandler, J.A. Taylor, Blood DNA methylation and breast Cancer: a prospective case-cohort analysis in the sister study, *J. Natl. Cancer Inst.* 112 (1) (2020) 87–94, <https://doi.org/10.1093/jnci/djz065>.
- [153] Y. Cheng, C. He, M. Wang, X. Ma, F. Mo, S. Yang, et al., Targeting epigenetic regulators for cancer therapy: mechanisms and advances in clinical trials, *Signal Transduct. Target. Ther.* 4 (2019) 62, <https://doi.org/10.1038/s41392-019-0095-0>.
- [154] R. Yang, K. Pfütze, M. Zucknick, C. Sutter, B. Wappenschmidt, F. Marne, et al., DNA methylation array analyses identified breast cancer-associated HYAL2 methylation in peripheral blood, *Int. J. Cancer* 136 (8) (2015) 1845–1855, <https://doi.org/10.1002/ijc.29205>.
- [155] J.H. Kim, M.H. Shin, S.S. Kwon, M.H. Park, J.H. Yoon, J.S. Lee, et al., Evaluation of promoter hypermethylation detection in serum as a diagnostic tool for breast carcinoma in Korean women, *Gynecol. Oncol.* 118 (2) (2010) 176–181, <https://doi.org/10.1016/j.ygyno.2010.04.016>.
- [156] M. Shan, H. Yin, J. Li, X. Li, D. Wang, Y. Su, et al., Detection of aberrant methylation of a six-gene panel in serum DNA for diagnosis of breast cancer, *Oncotarget* 7 (14) (2016) 18485–18494, <https://doi.org/10.18632/oncotarget.7608>.
- [157] B.P. de Almeida, J.D. Apolônio, A. Binnie, P. Castelo-Branco, Roadmap of DNA methylation in breast cancer identifies novel prognostic biomarkers, *BMC Cancer* 19 (1) (2019) 219, <https://doi.org/10.1186/s12885-019-5403-0>.
- [158] D. Nandy, S.M. Rajam, D. Dutta, A three layered histone epigenetics in breast cancer metastasis, *Cell Biosci.* 10 (2020) 52, <https://doi.org/10.1186/s13578-020-00415-1>.
- [159] M. Rohanizadeh, Analysis of circulating tumor DNA in breast cancer as a diagnostic and prognostic biomarker, *Cancer Genet.* 228–229 (2018) 159–168, <https://doi.org/10.1016/j.cancergen.2018.02.002>.
- [160] M. Agostini, M.V. Enzo, C. Bedin, V. Belardinelli, E. Goldin, P. Del Bianco, et al., Circulating cell-free DNA: a promising marker of regional lymph node metastasis in breast cancer patients, *Cancer Biomark.* 11 (2–3) (2012) 89–98, <https://doi.org/10.3233/CBM-2012-0263>.
- [161] L. Liu, L. Sun, C. Li, X. Li, Y. Zhang, Y. Yu, et al., Quantitative detection of methylation of FHIT and BRCA1 promoters in the serum of ductal breast cancer patients, *Biomed. Mater. Eng.* 26 (Suppl 1) (2015) S2217–22, <https://doi.org/10.3233/bme-151527>.
- [162] M. Berdasco, M. Esteller, Clinical epigenetics: seizing opportunities for translation, *Nat. Rev. Genet.* 20 (2) (2019) 109–127, <https://doi.org/10.1038/s41576-018-0074-2>.
- [163] P. Parrella, The value of epigenetic biomarkers in breast cancer, *Biomark. Med.* 12 (9) (2018) 937–940, <https://doi.org/10.2217/bmm-2018-0187>.
- [164] A. Goyal, R.K. Sahu, M. Kumar, S. Sharma, S. Qayyum, N. Kaur, et al., p16 promoter methylation, expression, and its association with estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 subtype of breast carcinoma, *J. Cancer Res. Ther.* 15 (5) (2019) 1147–1154, https://doi.org/10.4103/jcrt.JCRT_472_18.
- [165] M.L. Thomas, P. Marcato, Epigenetic modifications as biomarkers of tumor development, therapy response, and recurrence across the Cancer care continuum, *Cancers (Basel)* 10 (4) (2018), <https://doi.org/10.3390/cancers10040101>.
- [166] J. Martínez-Galán, B. Torres-Torres, M.I. Núñez, J. López-Peñalver, R. Del Moral, J.M. Ruiz De Almodóvar, et al., ESR1 gene promoter region methylation in free circulating DNA and its correlation with estrogen receptor protein expression in tumor tissue in breast cancer patients, *BMC Cancer* 14 (2014) 59, <https://doi.org/10.1186/1471-2407-14-59>.
- [167] A. Stone, E. Zotenko, W.J. Locke, D. Korbie, E.K.A. Millar, R. Pidsley, et al., DNA methylation of oestrogen-regulated enhancers defines endocrine sensitivity in breast cancer, *Nat. Commun.* 6 (2015) 7758, <https://doi.org/10.1038/ncomms8758>.
- [168] S. Mastoraki, A. Strati, E. Tzanikou, M. Chimoniou, E. Politaki, A. Voutsina, et al., ESR1 Methylation: A Liquid Biopsy-Based Epigenetic Assay for the Follow-up of Patients with Metastatic Breast Cancer Receiving Endocrine Treatment, *Clin. Cancer Res.* 24 (6) (2018) 1500–1510, <https://doi.org/10.1158/1078-0432.ccr-17-1181>.
- [169] N. Laham-Karam, G.P. Pinto, A. Poso, P. Kokkonen, Transcription and translation inhibitors in Cancer treatment, *Front. Chem.* 8 (2020) 276, <https://doi.org/10.3389/fchem.2020.00276>.
- [170] M. Swellam, M.D. Abdelmaksoud, M. Sayed Mahmoud, A. Ramadan, W. Abdel-Moneem, M.M. Hefny, Aberrant methylation of APC and RARβ2 genes in breast cancer patients, *IUBMB Life* 67 (1) (2015) 61–68, <https://doi.org/10.1002/iub.1346>.
- [171] T.C. de Ruijter, F. van der Heide, K.M. Smits, M.J. Aarts, M. van Engeland, V.C. G. Heijnen, Prognostic DNA methylation markers for hormone receptor breast cancer: a systematic review, *Breast Cancer Res.* 22 (1) (2020) 13, <https://doi.org/10.1186/s13058-020-1250-9>.
- [172] A.F. Vieira, F. Schmitt, An update on breast Cancer Multigene prognostic tests—emerging clinical biomarkers, *Front. Med. (Lausanne)* 5 (2018) 248, <https://doi.org/10.3389/fmed.2018.00248>.
- [173] Qiagen, QIAGEN Launches Predictive Therascreen PITX2 Test in Europe to Guide Treatment in High-risk Breast Cancer, 2018. https://corporate.qiagen.com/newsroom/press-releases/2018/20180206_pitx2_ce_ivd.
- [174] H.A. Abdel-Hafiz, K.B. Horwitz, Role of epigenetic modifications in luminal breast cancer, *Epigenomics* 7 (5) (2015) 847–862, <https://doi.org/10.2217/epi.15.10>.
- [175] K. Kamińska, E. Nalejska, M. Kubiak, J. Wojtyśiak, Ł. Żołna, J. Kowalewski, et al., Prognostic and predictive epigenetic biomarkers in oncology, *Mol. Diagn. Ther.* 23 (1) (2019) 83–95, <https://doi.org/10.1007/s40291-018-0371-7>.
- [176] Clinical trial 2020. <https://clinicaltrials.gov/>. (Accessed August 8th 2020).
- [177] C. Damaskos, N. Garmpis, S. Valsami, M. Kontos, E. Spartalis, T. Kalampokas, et al., Histone deacetylase inhibitors: an attractive therapeutic strategy against breast Cancer, *Anticancer Res.* 37 (1) (2017) 35–46, <https://doi.org/10.21873/anticancer.11286>.
- [178] A. Li, Z. Liu, M. Li, S. Zhou, Y. Xu, Y. Xiao, et al., HDAC5, a potential therapeutic target and prognostic biomarker, promotes proliferation, invasion and migration in human breast cancer, *Oncotarget* 7 (25) (2016) 37966–37978, <https://doi.org/10.18632/oncotarget.9274>.
- [179] S.R. Gameiro, A.S. Malamas, K.Y. Tsang, S. Ferrone, J.W. Hodge, Inhibitors of histone deacetylase 1 reverse the immune evasion phenotype to enhance T-cell mediated lysis of prostate and breast carcinoma cells, *Oncotarget* 7 (7) (2016) 7390–7402, <https://doi.org/10.18632/oncotarget.7180>.
- [180] T. Eckschlager, J. Plch, M. Stiborova, J. Hrabeta, Histone deacetylase inhibitors as anticancer drugs, *Int. J. Mol. Sci.* 18 (7) (2017), <https://doi.org/10.3390/ijms18071414>.
- [181] N. Garmpis, C. Damaskos, A. Garmpi, E. Kalampokas, T. Kalampokas, E. Spartalis, et al., Histone deacetylases as new therapeutic targets in triple-negative breast

- Cancer: progress and promises, *Cancer Genomics Proteomics* 14 (5) (2017) 299–313, <https://doi.org/10.21873/cgp.20041>.
- [182] P.N. Munster, K.T. Thurn, S. Thomas, P. Raha, M. Lacevic, A. Miller, et al., A phase II study of the histone deacetylase inhibitor vorinostat combined with tamoxifen for the treatment of patients with hormone therapy-resistant breast cancer, *Br. J. Cancer* 104 (12) (2011) 1828–1835, <https://doi.org/10.1038/bjc.2011.156>.
- [183] E.M. Newman, R.J. Morgan, S. Kummar, J.H. Beumer, M.S. Blanchard, C. Ruel, et al., A phase I, pharmacokinetic, and pharmacodynamic evaluation of the DNA methyltransferase inhibitor 5-fluoro-2'-deoxycytidine, administered with tetrahydrouridine, *Cancer Chemother. Pharmacol.* 75 (3) (2015) 537–546, <https://doi.org/10.1007/s00280-014-2674-7>.
- [184] G.O.S. Coyne, L. Wang, J. Zlott, L. Juwara, J.M. Covey, J.H. Beumer, et al., Intravenous 5-fluoro-2'-deoxycytidine administered with tetrahydrouridine increases the proportion of p16-expressing circulating tumor cells in patients with advanced solid tumors, *Cancer Chemother. Pharmacol.* 85 (5) (2020) 979–993, <https://doi.org/10.1007/s00280-020-04073-5>.
- [185] R.M. Connolly, H. Li, R.C. Jankowitz, Z. Zhang, M.A. Rudek, S.C. Jeter, et al., Combination epigenetic therapy in advanced breast Cancer with 5-Azacidine and entinostat: a phase II national Cancer institute/stand up to Cancer study, *Clin. Cancer Res.* 23 (11) (2017) 2691–2701, <https://doi.org/10.1158/1078-0432.ccr-16-1729>.
- [186] A.T. Lorincz, The promise and the problems of epigenetics biomarkers in cancer, *Expert Opin. Med. Diagn.* 5 (5) (2011) 375–379, <https://doi.org/10.1517/17530059.2011.590129>.
- [187] A. Roberti, A.F. Valdes, R. Torrecillas, et al., Epigenetics in cancer therapy and nanomedicine, *Clin. Epigenet.* 11 (1) (2019) 81, <https://doi.org/10.1186/s13148-019-0675-4>.