Epigenetic Therapy in Breast Cancer

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Abstract Breast carcinogenesis is a multistep process involving both genetic and epigenetic changes. Epigenetics is defined as reversible changes in gene expression, not accompanied by alteration in gene sequence. DNA methylation, histone modification, and nucleosome remodeling are the major epigenetic changes that are dysregulated in breast cancer. Several genes involved in proliferation, anti-apoptosis, invasion, and metastasis have been shown to undergo epigenetic changes in breast cancer. Because epigenetic changes are potentially reversible processes, much effort has been directed toward understanding this mechanism with the goal of finding effective therapies that target these changes. Both demethylating agents and the histone deacetylase inhibitors (HDACi) are under investigation as single agents or in combination with other systemic therapies in the treatment of breast cancer. In this review, we discuss the role of epigenetic regulation in breast cancer, in particular focusing on the clinical trials using therapies that modulate epigenetic mechanisms.

Keywords Breast cancer · Epigenetic targets · Demethylating agents · Histone deacetylase inhibitors

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Introduction

The modern definition of epigenetics is heritable or transient changes in gene expression without a specific change in the DNA sequence [1]. Over the past decade, a tremendous explosion of research in the area of epigenetics and cancer has resulted in a much better understanding of the various mechanisms and effects underlying these non-structural changes to the genome. The exciting aspect of discovering various epigenetic alterations is the potential opportunity to reverse the effects with therapies that can result in gene re-expression. DNA methylation and histone modification resulting in nucleosome remodeling lead to transcriptional silencing of critical tumor-suppressor and growth-regulatory genes in breast cancer. Much of the preclinical work has focused upon using epigenetic therapies to re-express the silenced gene in cell lines. The more challenging aspect has been the clinical application of what we see in the laboratory and clinical effectiveness of such a strategy. For example, preclinical work clearly demonstrates that the maspin gene can be re-expressed in breast cancer cell lines using demethylating agents and histone deacetylase inhibitors (HDACi), but the crucial question is whether even if such a re-expression was achieved in a patient's tumor, would it have any impact on clinical outcomes [2]. Despite these challenging aspects of the clinical application of epigenetic therapy, advances have been made in exploring and identifying the potential role of such therapies in breast cancer. At present though, all of the epigenetic therapies are in investigational stages in solid tumors and not yet considered standard of care. The areas where we are gaining more knowledge about the potential use of these agents in breast cancer are as combination with targeted therapies, chemotherapy, and radiation to overcome resistance and improve sensitivity to treatment

(Fig. 1). Another exciting area in early development is the role of epigenetic modulation in prevention of breast cancer. In this review, we summarize the key epigenetic targets and give an overview of the completed and ongoing preclinical and clinical studies with epigenetic therapies in breast cancer.

Principles of Epigenetics

DNA Methylation

The role of DNA methylation in the epigenetic regulation of human breast cancer has been well characterized. Hypermethylation of CpG islands, which are CpG dinucleotide-rich regions located in the 5′ end of region of genes, commonly occurs in cancer cells [3]. Poorly differentiated breast tumors have more methylated CpG islands than moderately or well-differentiated tumors in breast cancer [4]. Hypermethylated CpG dinucleotides within promoter regions play an important role in regulation of individual genes that promote carcinogenesis. In breast cancer, promoter hypermethylation and subsequent gene silencing occurs in estrogen response genes (ESR1, PR), proapoptotic genes (HOXA5, TMS1), cell cycle–inhibitor genes (p16, RASSF1A), DNA repair genes (BRCA1), and many others [5]. Advances in technologies that assess genome-wide DNA methylation patterns are an important step toward understanding the complex role of this process in malignancy [6].

DNA methylation is mediated by the action of DNA methyltransferases (DNMTs). There are three active DNA methyltransferases: DNMT1, DNMT3a, and DNMT3b. DNMT1 and 3B show increased expression in breast cancer [7]. DNMTs directly interact with histone deacetylases (HDACs) and the methyl-CpG-binding domain (MBD) family of proteins at the promoter regions to form a repressive transcription complex [8]. This complex is important in the conversion of acetylated histones to the deacetylated inactive form. As discussed in subsequent sections, DNA methylation, histone modification, and subsequent nucleosome remodeling are coordinated processes that can be targeted in breast cancer therapeutics [9].

Post-translational Histone Modification

Epigenetic regulation of gene transcription relies on the cooperative mechanisms of DNA methylation and histone acetylation. Histone modification and overall chromatin structure are governed by balanced counteractivity of histone acetyltransferases (HAT) and HDAC. Increased acetylation of histones, which promotes a more open chromatin, is associated with gene activation. In addition, histone methyltransferases (HMTs) mediate histone methylation. Recently, distinct patterns in post-translational histone modification were observed in breast cancer–specific subtypes. Higher global histone acetylation and histone methylation have been observed in the more favorable luminal A subtype, and decreased levels have been seen in the basal subtype [10].

Post-translational histone modifications include the loss of acetylation in lysine 16 and trimethylation of lysine 20 in histone H4 [11]. In addition, two major histone methylation patterns have been identified: 1) methylation of lysine residue 9 on the core histone protein H3, resulting in gene silencing; and 2) methylation of lysine 4 and recently lysine 27 on H3, resulting in activated genes [12]. In addition, the

Fig. 1 Application of epigenetic therapies in breast cancer
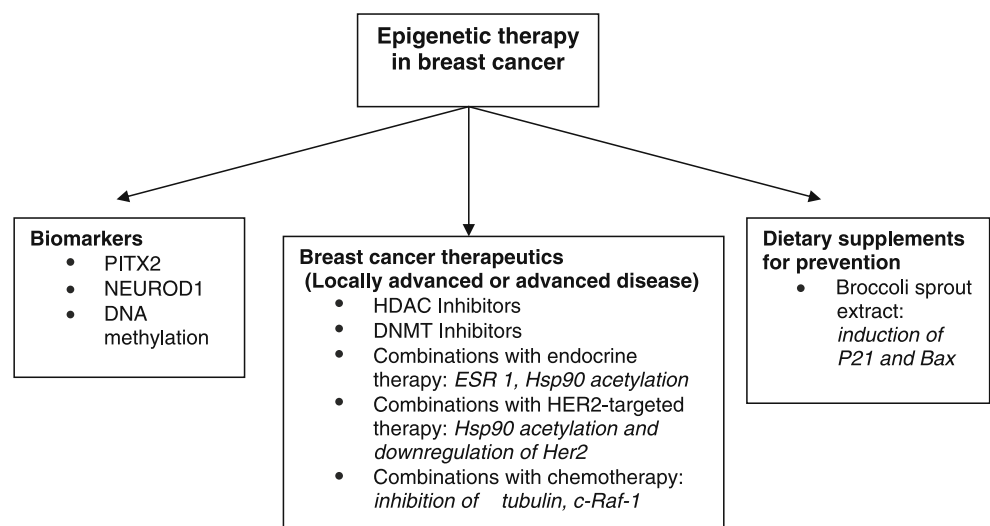
loss of trimethylation of lysine 27 of H3 (H3K27) has been shown to be a negative prognostic indicator in several cancers, including breast cancer [12]. Histone and nucleosome conformational changes are also regulated by the chromatin remodeling complex [13]. These intricate mechanisms that regulate histones are potentially modifiable by epigenetic therapy, including with HDACi therapy.

Known Epigenetic Changes in Breast Cancer

A number of epigenetic changes have been described in breast cancer. Epigenetic changes are not limited to CpG islands and histone post-translational modifications but also include microRNA (miRNAs) [14•]. Aberrant hypermethylation of mir-9-1, mir-124a3, mir-148, mir-152, and mir-663 is present in primary breast tumor specimens [14•]. Additional epigenetic changes in breast cancer include genetic instability with aneuploidy and telomere erosion, which may be associated with epigenetic modifications [15]. The tumor microenvironment may also undergo epigenetic changes that regulate the transition of in situ to invasive breast cancer [16]. Although it is beyond the focus of this review to summarize all of the known epigenetic changes in breast cancer, we can focus on the epigenetic changes in some the key genes involved and their clinical implications.

Epigenetic Regulation of ER

More than two thirds of breast cancers are positive for estrogen receptor (ER) expression at the time of diagnosis and have the potential to benefit from endocrine therapy. However, both de novo and acquired resistance to endocrine therapy pose significant clinical challenges. Response to endocrine therapy is proportional to the degree of ER expression, and loss of ER expression over time is one of the major mechanisms underlying endocrine resistance. A better understanding of the epigenetic regulation of ER presents opportunities for devising rational epigenetic therapies to reverse endocrine resistance to tamoxifen as well as other endocrine therapies, such as aromatase inhibitors. This also provides novel opportunities to treat ER-negative tumors by epigenetic modulation (leading to ER re-expression) and hormonal therapy.

The role of epigenetic changes in the regulation of ER is well established. In brief, the epigenetic regulation of ER is mediated though the recruitment of multimolecular complexes containing HDAC1 and DNMT1 that are recruited to the promoter region as well as other members of the corepressor complex [17]. DNMTl is highly expressed in ER-negative cells throughout the cell cycle. In addition to DNMTs, the interaction of deacetylated histones with the methylated ER gene promoter is a prerequisite for its inactivation. Based on this biologic rationale, there is a synergistic interaction between DNMT inhibitors and HDACi in the induction of ER expression in ER-negative cell lines [18].

Interestingly, HDACi alone can also restore ER expression via chromatin remodeling [19]. Further evidence for the role of histone post-translational modification in ER regulation comes from studies showing that SET7, an HMT that is known to monomethylate H3K4, stabilizes ER, which increases sensitivity to estrogens and enhances transcription of estrogen-response elements [20]. This suggests that post-translational modification of ER may play a role in hormone responsiveness, and hence presents a target for epigenetic therapy with HDACi.

The epigenetic silencing of ER target genes is crucial to the development of ER- independent growth. As a consequence of ER inactivation, the downstream ER targets are silenced by the recruitment of repressor complexes to the promoters of the target genes [21]. A number of preclinical studies have shown that expression of ER can be impacted by epigenetic therapy. Collectively, these data are the basis for the biological rationale for combining hormone therapy with epigenetic-targeted therapies. These trials are discussed in subsequent sections.

Epigenetic Regulation of PR

Similarly to ER, progesterone receptor (PR) expression levels are regulated by epigenetic mechanisms that may impact endocrine therapy responsiveness. A CpG island is located in the first exon of the PR gene, and PR-negative tumors have higher rates of PR promoter hypermethylation [22]. Demethylation of the CpG island is required for the ER binding to this regulatory sequence [23]. In ER-negative tumors, silencing of the ER gene also affects downstream estrogen-regulated gene expression, including PR expression [23]. In vitro, long-term exposure to tamoxifen inhibits PR expression via epigenetic mechanisms, including chromatin remodeling [24].

Epigenetic Regulation of HER2 Neu

Human epidermal growth factor receptor 2 (HER2) neu–overexpressing tumors have higher numbers of genes with methylated CpG islands [25]. In addition, methylation of specific genes, including those for H-cadherin (CDH-13), PR, and 17-B-hydroxysteroid dehydrogenase (important in ER metabolism) correlate with HER2 amplification [26]. Synergistic interaction of the HDACi vorinostat with trastuzumab has been shown in Her2-positive cell lines [27]. Similarly, the HDACi LAQ824 sensitizes human breast cancer cells to trastuzumab via downregulation of HER2 [28].

Biologic Rationale of DNMT Inhibitor Therapy

Preclinical Data with DNMT Inhibitors

The two most commonly investigated DNMT inhibitors are nucleoside analogues 5-azacytidine (5-aza) and 5-aza-2′-deoxycytidine (decitabine). Treatment of ER-negative human breast cancer cell lines with 5-aza promotes the partial demethylation of the ER CpG island, re-expression of ER mRNA, and synthesis of functional ER protein [29]. Treatment with 5-aza results in the increased expression of *mir*-9-1 [14•]. Inhibition of DNMT1 by antisense, oligo, or siRNA may increase estrogen responsiveness in ER-negative breast cells and increase responsiveness to 5-aza [30]. As described earlier, the addition of HDACi to DNMT inhibitors is synergistic [18].

Clinical Studies with Single-agent DNMT Inhibitors

Currently, no data are available on the clinical activity of 5-aza or decitabine as single agent in breast cancer. A phase I multicenter trial of decitabine in patients with advanced solid tumor malignancies including breast cancer who had failed standard therapy was recently completed (NCT00030615) (http://clinicaltrials.gov). Results of the trial are not yet available. Other nucleoside analogue DNMT inhibitors such as zebularine and 5-fluoro-2′-deoxycitine are in clinical development [31]. An anti-sense oligonucleotide that specifically targets DNMT1 called MG98 is also in clinical development.

Clinical Studies of DNMT Inhibitors with HDACi

A phase I study of the combination of decitabine and HDACi vorinostat given sequentially in advanced solid tumors showed disease stabilization in seven of 22 evaluable patients, including two patients with breast cancer. Dose-limiting toxicities (DLT) include myelosuppression and gastrointestinal toxicities [32•]. A proof-of-principle phase I study was conducted to study the effects of addition of hydralazine and magnesium valproate to neoadjuvant doxorubicin and cyclophosphamide for locally advanced breast cancer [33]. Eligible patients received oral hydralazine and valproate starting day 7 through completion of four cycles of chemotherapy. Core biopsies performed on day 8 of this combination epigenetic therapy showed significant reduction of the global methylation, and HDAC inhibition was observed in peripheral blood mononuclear cells (PBMCs). The combined chemotherapy and epigenetic therapy was generally well tolerated, and increased incidence of drowsiness was attributed to the addition of valproate to the regimen. Although most patients had a clinical response, only one (6.6%) patient

had a complete pathologic response. This proof-of-concept study led to a phase II single-arm study of hydralazine and magnesium valproate added to the same chemotherapy on which patients (with advanced solid tumors) were progressing to overcome chemotherapy resistance [34]. Three patients with breast cancer were treated on this study. Two patients who had progressed on paclitaxel were continued on the same along with hydralazine and magnesium valproate and both progressed. Another patient was continued on the same failed hormone therapy along with the combined epigenetic therapies and had stable disease for 4.5 months. Although responses were seen in a few patients with genitourinary tumors, this approach has not been developed further in breast cancer.

Biologic Rationale of HDACi Therapy

Mechanisms of Action of HDACi

HDACi are a class of drugs with broad anti-proliferative and pro-apoptotic effects in a variety of malignant cell types [3]. The only HDACi that is currently approved by the US Food and Drug Administration is vorinostat (suberoylanilide hydroxamic acid) for the treatment of cutaneous T-cell lymphoma. HDACi promote hyperacetylation of histones, which leads to the transcriptional upregulation of pro-apoptotic genes and cyclin-dependent kinase inhibitors such as p21 and p27. In addition, HDACi increase acetylation of the Hsp90 chaperone complex, leading to the destabilization [35]. As a result, proteosome targeting and degradation of multiple client proteins, including HER2, ER, AKT and c-Raf, are increased. Downstream effects of client protein degradation affect a myriad of pathways, including dose-dependent increase of pro-apoptotic proteins Bak and Bim and attenuation of anti-apoptic proteins Bcl-2 and Bcl-x_L [36]. In preclinical models, ER is one of the Hsp90 client proteins that are most sensitive to Hsp90 inhibition [37]. By attenuating these downstream signaling pathways, HDACi sensitize breast cancer cells to endocrine, HER2-directed, and cytotoxic therapy.

Preclinical Data with HDACi

Vorinostat is part of the hydroxamic acid family of HDACi and inhibits the proliferation of both ER-positive and ER-negative human breast cancer cell lines [38]. Exposure to vorinostat and another HDACi, LAQ824, downregulates p-Akt, Akt, and c-Raf, sensitizing ER-positive breast cancer cells to tamoxifen [39]. Selective inhibition of HDAC2 by siRNA downregulates both ER and PR expression and potentiates the effects of tamoxifen in ER-positive breast

tumor cells [40]. Furthermore, the combination of vorinostat with trastuzumab or docetaxel is synergistic in breast cancer cells with attenuated levels of c-Raf and AKT [36]. Prolonged exposure to vorinostat also sensitizes breast cancer cells to topoisomerase inhibitors [41]. Based on the promising preclinical data, there are multiple clinical trials in breast cancer investigating the effects of vorinostat in combination with endocrine therapy and cytotoxic agents.

Clinical Studies with Single-agent HDACi Therapy

A phase I trial of vorinostat administered intravenously in patients with advanced solid tumor and hematologic malignancies was well tolerated. Post-therapy tumor biopsies showed the accumulation of acetylated histones [42]. No partial responses (PR) or complete responses (CR) were seen, but there was radiographic evidence of tumor regression in two patients with lymphoma and two patients with bladder cancer. A subsequent phase I trial using oral vorinostat was completed showing one (1%) CR and three (4%) PR [43]. Histone acetylation was present in PBMCs, which persisted beyond the half-life of vorinostat. In the phase II study conducted in advanced breast cancer with single-agent oral vorinostat (200 mg twice daily for 14/21 days), 4 of 14 patients (29%) achieved stabilization of disease (SD) (range, 4–14 months) but no patients had objective responses. The most common toxicities included fatigue, nausea, diarrhea, and lymphopenia. Due to the lack of objective response, the study was not continued beyond the first stage [44].

Combination Studies of HDACi and Endocrine Therapy

Based on promising preclinical data of combining HDACi with endocrine therapy, a phase II study of vorinostat in combination with tamoxifen was conducted in metastatic breast cancer patients who had progressed on previous lines of hormone therapy [45•]. Seven patients (21%) had a confirmed PR and one with bone-only disease had an objective response by PET/CT, whereas another four (12%) patients had SD of >6 months in duration. The toxicity profile of the combination was acceptable with pulmonary emboli (5%) in two patients. The median response duration was 8 months. Correlative studies revealed histone H3 and H4 acetylation at day 8, suggesting adequate vorinostat plasma levels in the majority of the patients.

Preliminary results from a phase II study of a benzamide HDACi, entinostat, suggest a potential role for reversal of aromatase inhibitor (AI) endocrine therapy resistance [46]. Eligible postmenopausal women who had progressed on an AI were continued on the same AI they were receiving along with entinostat at 5 mg weekly on a 28-day cycle. One patient had a confirmed PR and one patient had SD>

6 months. Preliminary biomarker analysis indicated that with the addition of HDACi therapy there was increased lysine acetylation and increased apoptosis in PBMCS. A phase II, randomized, double-blind, multicenter study of exemestane with and without entinostat in postmenopausal women with locally recurrent or metastatic ER-positive breast cancer progressing on treatment with a nonsteroidal AI is currently ongoing (http://clinicaltrials.gov).

Combination Studies of HDACi and Chemotherapy

In breast cancer, several trials are ongoing, including multicenter phase I/II trials with the addition of vorinostat to paclitaxel and bevacizumab in metastatic breast cancer [47•]. Interim analysis reported that among 45 evaluable patients, 24 (53%) patients had an objective response and 11 patients had SD, with 5 patients with SD≥24 weeks. The median time to progression was 13.8 months. Two patients had tumor biopsies, and PBMCs collected before and 4 h after the third dose of vorinostat showed increased acetylation of K69 lysine residue of the chaperone protein Hsp 90, upregulation of Hsp70, and downregulation of AKT by Western Blot, providing evidence of Hsp90 inhibition in tumors and PBMCs. Final analysis of an eight-patient cohort with mandatory before and after treatment biopsies is in process. Additional ongoing studies with vorinostat and other cytotoxic agents are noted in Tables 1 and 2.

Other combination studies of HDACi with chemotherapy include a phase I trial of valproic acid followed by epirubicin in advanced, heavily pretreated solid tumors. This combination was well tolerated and had anti-tumor activity [48]. In the dose-expansion part of this study, 15 patients with breast cancer received valproic acid with FEC 100 (5-fluorouracil, epirubicin, and cyclophosphamide). Objective responses were seen in nine of 14 (64%) evaluable patients. Based on promising results of the phase I study, currently a phase II neoadjuvant trial of valproic acid with FEC 100 in locally advanced breast cancer is ongoing (NCT 00437801) (http://clinicaltrials.gov).

Combination Studies of HDACi and HER2-Targeted Therapy

Based on the preclinical rationale of downregulation of HER2 by HDACi, a phase I/II study of vorinostat in combination with trastuzumab in patients with Her2-positive advanced breast cancer resistant to trastuzumab-containing regimens was conducted [49]. Eligible patients were those with measurable disease who had failed prior trastuzumab and could have received prior lapatinib. Sixteen patients were enrolled, of which only 10 were centrally confirmed to have Her2-positive disease (5 were

Table 1 Completed epigenetic clinical trials in breast cancer

Epigenetic category	Epigenetic drug	Additional agents	Type of breast cancer	Type of study	Status/results	Reference
DNMT inhibitors	Decitabine	None	Advanced solid tumors	Phase I	Completed. No results reported.	(http://clinicaltrials.gov)
DNMT inhibitors+ HDAC inhibitors	Decitabine	Vorinostat	Advanced solid tumors	Phase I	N=22 7 (32%) SD	[32•]
HDAC inhibitors	Vorinostat (intravenous)	None	Advanced solid tumors and hematologic malignances	Phase I	N=37 0 CR 0 PR 4 patients with objective tumor regression	[42]
	Vorinostat (orally)	None	Advanced solid tumors	Phase I	N=73 1 (1%) CR; 3 (4%) PR	[43]
	Vorinostat	None	Advanced breast cancer	Phase II	N=14 0 CR; 0 PR; 4 (29%) SD	[44]
HDAC inhibitors+ endocrine therapy	Vorinostat	Tamoxifen	Advanced, ER+	Phase II	N=43; 34 evaluable 7 (21%) PR; 4 (12%) SD	[45•]
	Entinostat (SNDX-275)	Aromatase inhibitor	Advanced, ER+	Phase II	N=27 1 (37%)PR; 1 (37%) SD	[46]
HDAC inhibitors+ chemotherapy	Hydralazine+ valproic acid	Doxorubicin, Cytoxan	Locally advanced breast cancer; neoadjuvant	Proof of principle, phase I	N=15 1 (6.6%) complete pathologic response	[33]
	Hydralazine+ valproic acid	Various agents	Advanced solid tumors	Phase II	N=3 (breast cancer) 1 (33%) SD	[34]
	Vorinostat	Paclitaxel, bevacizumab	Advanced; First-line chemotherapy	Phase I/II	N=55; 45 evaluable 24 (53%) objective response	(http://clinicaltrials.gov)
	Valproic acid	FEC 100	Advanced solid tumors	Phase I	N=14 (breast cancer) 9 (64%)	[48]
HDAC inhibitors+ HER2- targeted therapies	Vorinostat	Trastuzumab	Advanced breast cancer	Phase II	N=11 (HER2+) No objective responses	[49]

CR complete response, ER estrogen receptor, HDAC histone deacetylase, DNMT DNA methyltransferase, PR partial response, SD stable disease

Her2 negative and 1 had insufficient tissue for marker analysis). None of the 11 patients (Her2 positive or unknown) had an objective response. However, one patient with centrally confirmed HER2-negative disease had an objective response. The preliminary data indicate that the addition of HDACi to trastuzumab in heavily pretreated patients was not associated with a response to support a reversal of trastuzumab resistance. As shown in Table 1, additional studies with vorinostat are planned with combination of chemotherapy and trastuzumab and with lapatinib.

A phase I/II multicenter study of pan-HDAC inhibitor, panobinostat, with trastuzumab in metastatic breast cancer patients who have failed trastuzumab is currently ongoing

(NCT00567879) and preliminary results have been reported [50]. In the first cohort, two patients with liver metastasis experienced tumor reduction of 29%, and four patients received over eight cycles of the combination. These preliminary results demonstrate that the combination of panobinostat and trastuzumab shows promising activity. Another study with the addition of paclitaxel to this regimen is currently ongoing (NCT00788931) (http://clinicaltrials.gov). Preliminary results from a phase I study of panobinostat with lapatinib with or without capecitabine were recently presented showing tolerability and promising activity in the combination regimen [51]. Additional ongoing studies with HDACi and HER2-targeted therapies are summarized in Tables 2 and 3.

Table 2 Ongoing epigenetic clinical trials in breast cancer

Epigenetic category	Epigenetic drug	Additional agents	Type of breast cancer	Type of study	Status	Reference
DNMT inhibitors+ chemotherapy	5-aza	Nab-Paclitaxel	Advanced	Phase I/II	Recruiting	NCT00748553 (http://clinicaltrials.gov)
HDAC inhibitors	Vorinostat	None	Stage I–III, neoadjuvant	Biomarker	Active (not recruiting)	NCT00262834 (http://clinicaltrials.gov)
	Vorinostat	None	Ductal carcinoma in situ	Biomarker	Recruiting	NCT00788112 (http://clinicaltrials.gov)
	Valproic acid	None	Stage II–III, neoadjuvant	Biomarker	Recruiting	NCT01007695 (http://clinicaltrials.gov)
	Panobinostat	None	Advanced, ER+ tamoxifen refractory	Biomarker	Recruiting	NCT00993642 (http://clinicaltrials.gov)
HDAC inhibitors+ endocrine therapy	Entinostat (SNDX-275)	Exemestane	Advanced, ER+	Phase II	Recruiting	NCT00676663 (http://clinicaltrials.gov)
	Panobinostat	Letrozole	Advanced, ER+	Phase I/II	Recruiting	NCT01105312 (http://clinicaltrials.gov)
HDAC inhibitors+ chemotherapy	Vorinostat	Capecitabine	Advanced, up to 2 prior chemotherapy	Phase I/II	Recruiting	NCT00719875 (http://clinicaltrials.gov)
	Vorinostat	Ixabepilone	Advanced	Phase I	Recruiting	NCT01084057 (http://clinicaltrials.gov)
	Vorinostat	Nab-paclitaxel, carboplatin	Neoadjuvant chemotherapy	Phase II		NCT00616967 (http://clinicaltrials.gov)
	Valproic acid	FEC100	Neoadjuvant chemotherapy	Phase II	Recruiting	NCT00437801 (http://clinicaltrials.gov)
HDAC inhibitors+HER2-targeted therapy	Vorinostat	Paclitaxel, trastuzumab	Neoadjuvant chemotherapy	Phase I/II	Recruiting	NCT00574587 (http://clinicaltrials.gov)
	Vorinostat	Lapatinib	Advanced, HER2+	Phase II	Recruiting	NCT01118975 (http://clinicaltrials.gov)
	Panobinostat	Trastuzumab	Advanced, HER2+	Phase I	Recruiting	NCT00567879 [50]
	Panobinostat	Paclitaxel, trastuzumab	Advanced, HER2+	Phase I/II	Recruiting	NCT00788931 (http://clinicaltrials.gov)
	Panobinostat	Lapatinib± capecitabine	Advanced, HER2+	Phase I	Recruiting	NCT00632489 [51]

5-aza 5-azacytidine, *DNMT* DNA methyltransferase, *ER* estrogen receptor, *HDAC* histone deacetylase

Table 3 Epigenetic drugs

Drug class	Agents	Phase of development in breast cancer	Current clinical approval	Reference
DNMT inhibitors	5-aza	Phase I in combination with chemotherapy	Myelodysplastic syndromes	NCT00748553 (http://clinicaltrials.gov)
	Decitabine	Phase I study completed	Myelodysplastic syndromes	[30]
HDAC inhibitors	Vorinostat	Phase I/II single agent or in combination with chemotherapy and HER2-targeted therapies	Cutaneous T-cell lymphoma	NCT00748553 (http://clinicaltrials.gov) NCT00262834 (http://clinicaltrials.gov) NCT00719875 (http://clinicaltrials.gov) NCT00574587 (http://clinicaltrials.gov) NCT01118975 (http://clinicaltrials.gov)
	Valproic acid±hydralazine	Phase I and phase II completed. No additional studies planned.	Neuropsychiatric indications; investigational only in cancer therapy	[32•, 34]
	Panobinostat	Phase I/II studies with endocrine therapy, HER2-targeted therapy, and chemotherapy	Investigational only	NCT01105312 (http://clinicaltrials.gov) NCT00567879 [50] NCT00788931 (http://clinicaltrials.gov) NCT00632489 [51]
	Entinostat (SNDX-275)	Phase II combination with exemestane	Investigational only	NCT00676663 (http://clinicaltrials.gov)

5aza 5-azacytidine, *DNMT* DNA methyltransferase, *HDAC* histone deacetylase

Epigenetic Therapy as a Chemopreventive Strategy

Sulforaphane is an isothiocyanate present in abundance in broccoli sprouts and has been shown to exert anti-cancer effects through histone acetylation, induction of *P21* and *Bax*, and induction of cell cycle arrest and apoptosis. Ingestion of 1 cup of broccoli inhibited HDAC activity in PBMCs 3–6 h after consumption, resulting in induction of H3 and H4 acetylation, providing rationale to use this dietary ingredient as a chemopreventive agent. Based on this, a phase II randomized, placebo-controlled, double-blind trial of broccoli sprout extract in women with newly diagnosed ductal carcinoma in situ and/or atypical ductal hyperplasia is currently ongoing (NCT00843167) (http://clinicaltrials.gov). Results from this study may establish a role for epigenetic therapy in prevention of breast cancer.

Epigenetic Prognostic and Predictive Factors in Therapy

Epigenetic changes have been investigated as both prognostic and predictive factors in breast cancer. A number of epigenetic changes have been investigated as prognostic factors. For example, DNA hypermethylation of PITX2 is a marker of poor prognosis in node-negative, hormone receptor–positive breast cancer after adjuvant tamoxifen therapy [52]. Promoter methylation of the NEUROD1, a member of the stem cell, polycom group oftarget genes, has been investigated as a marker for chemosensitivity in the neoadjuvant setting [53]. Most recently, investigators from Europe reported on the first large-scale epigenotyping study of the peripheral blood DNA and demonstrated that DNA methylation provides a good prediction of breast cancer risk that is not completely independent of traditional risk factors for breast cancer [54•].

A variety of methods have been used by different studies to evaluate methylation patterns, including methyl-specific polymerase chain reaction, MethyLight, and bisulfite pyrosequencing with lack of standardization across groups, which has made it challenging to compare results across studies. With advances in high-throughput technology for assessment of genome-wide methylation patterns such the MALDI-TOF mass array, additional hypermethylated CpG islands have been identified in breast cancer [55•].

The challenge remains as to how to best define optimal biological endpoints with epigenetic therapy. Because the epigenetic changes induced by DNMTs and HDACi are transient and reversible, it particularly important to have tissue-specific measures of whether epigenetic targets are effectively impacted by therapy. As discussed in the previous section, several trials have utilized PBMCs for evaluation of H3 and H4 acetylation levels as well as the AKT pathway. Pretreatment and post-treatment tissue biopsies remain the best way to assess pharmacodynamics' epigenetic effects. The phase I/II study with addition of vorinostat to paclitaxel and bevacizumab has an expanded cohort of eight patients with required tumor biopsies before and after treatment. Results are pending from this portion of the study. However, serial biopsies are not always readily possible in many women with breast cancer due to inaccessibility of tumor sites and patient preference [47•]. Furthermore, even if we do establish pharmacodynamic endpoints that can be easily assessed, no studies have established the clinical relevance of meeting these endpoints. The search is also ongoing for validated predictive biomarkers that can help to identify tumors that are particularly sensitive to epigenetic modulation.

Conclusions

Targeting epigenetic changes in breast cancer is an exciting and evolving arena that has the potential to change our current standard-of-care therapies. In this review, we have summarized the complex epigenetic regulatory pathways that can impact breast cancer prognostication and therapeutics. Particularly promising is the combination of epigenetic therapy with our existing cytotoxic and endocrine therapies in breast cancer. However, many uncertainties still remain as to how to best translate these findings in the clinical arena. Given that the epigenetic changes induced by DNMTs and HDACi are transient and reversible, a number of studies are ongoing to help define the optimal doses and treatment schedules for these agents.

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