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The epigenetics of breast cancer – Opportunities for diagnostics, risk stratification and therapy

Rieke Schröder[a], Anna-Lena Illert[b], Thalia Erbes[c], Christian Flotho[a,d], Michael Lübbert[b,d], and Jesús Duque-Afonso [b]

[a]Department for Pediatric Hematology and Oncology, Faculty of Medicine and University of Freiburg Medical Center, University of Freiburg, Freiburg, Germany; [b]Department of Hematology/Oncology/Stem Cell Transplantation, University of Freiburg, Freiburg, Germany; [c]Department of Gynecology, Faculty of Medicine and University of Freiburg Medical Center, University of Freiburg, Freiburg, Germany; [d]German Cancer Consortium (Deutsches Konsortium Für Translationale Krebsforschung, DKTK), Freiburg, Germany

ABSTRACT

The stage and molecular pathology-dependent prognosis of breast cancer, the limited treatment options for triple-negative carcinomas, as well as the development of resistance to therapies illustrate the need for improved early diagnosis and the development of new therapeutic approaches. Increasing data suggests that some answers to these challenges could be found in the area of epigenetics. In this study, we focus on the current research of the epigenetics of breast cancer, especially on the potential of epigenetics for clinical application in diagnostics, risk stratification and therapy. The differential DNA methylation status of specific gene regions has been used in the past to differentiate breast cancer cells from normal tissue. New technologies as detection of circulating nucleic acids including microRNAs to early detect breast cancer are emerging. Pattern of DNA methylation and expression of histone-modifying enzymes have been successfully used for risk stratification. However, all these epigenetic biomarkers should be validated in larger clinical studies. Recent preclinical and clinical studies show a therapeutic benefit of epigenetically active drugs for breast cancer entities that are still difficult to treat (triple negative, UICC stage IV). Remarkably, epigenetic therapies combined with chemotherapies or hormone-based therapies represent the most promising strategy. At the current stage, the integration of epigenetic substances into established breast cancer therapy protocols seems to hold the greatest potential for a clinical application of epigenetic research.

Introduction

Mammography screening offers an established and accessible system for the early detection of asymptomatic breast cancer [1]. At diagnosis, immunohistochemical classification allows for therapeutic and prognostic guidelines. However, despite the establishment of complex therapeutic regimes, the prognosis of breast cancer remains dependent on stage and molecular pathology. In addition, the spectrum of treatment options is still very limited, especially for triple-negative breast cancer (TNBC) and metastatic diseases [2]. Encouragingly, there is increasing evidence that answers to these clinically highly relevant challenges could be found in the field of epigenetics.

Method

A selective literature search for publications in breast cancer and epigenetics research was conducted via PubMed and GoogleScholar, supplemented by a reference search. Included were articles published from 1960 until August 2020 in English language. The search was based on the keywords 'epigenetics,' 'breast cancer,' 'DNA methylation,' 'histone acetylation,' 'histone methylation.' Clinical trials were identified via the web-

sites of the registry studies clinicaltrials.gov, drks. de, cancer.gov.

Results

Epigenetics

In contrast to the term genetics, epigenetics describes changes in gene expression that do not originate from a modification of the DNA nucleotide sequence [3]. Instead, gene regulatory information is stored in the form of biochemical modifications of cytosine bases and histone proteins [4].

According to current understanding, epigenetic modifications of the chromatin structure take place at the amino acid residues of histone tails, which provide a free surface for potential protein-protein interactions. This regulatory function, inherent in their chemical structure, depends on their abundance [5].

DNA methylation is a covalent modification of post-replicative DNA that creates a 'fifth base' from cytosine: 5-methylcytosine (5-mC). This modification takes place mainly in parts of the genome known as CpGs, where a cytosine base is followed by a guanine nucleotide. This methylation is catalysed by DNA methyltransferases (DNMT) [6].

Epigenetics of breast cancer

Screening and early diagnosis

The prognosis of each individual with diagnosed breast cancer is strongly dependent on the stage and molecular pathology of the disease, which highlights the need for continuous improvement in screening and early diagnosis.

In a comparative methylation analysis of tissue samples from healthy controls, Ductal Carcinoma in situ (DCIS) and invasive breast cancer, Fleischer et al. showed radical changes within the methylation profiles from one cellular disease progression to the next [7]. Interestingly, the majority of methylation changes, both increasing and decreasing, occurred during the progression from healthy breast tissue to DCIS. In comparison, the change in methylation pattern from DCIS to invasive breast cancer appeared to be rather marginal.

This further supports the hypothesis, that methylation changes play an early role in the carcinogenesis of breast cancer and thus represent a reasonable target for improving early diagnosis [8].

Recent research has established the so-called Differentially Methylated Regions (DMRs) as potential biomarkers. They describe genetic regions, mostly CpGs, whose methylation patterns show significant differences between healthy breast tissue and breast cancer cells [9,10]. The gene panels based on these analyses distinguished between physiological control samples and breast cancer samples within the study cohorts. However, the respective DMRs or genes examined differed considerably from publication to publication and showed only very few overlaps [11–14]. In addition, the largest current meta-analysis of methylation patterns in blood samples from healthy subjects found no evidence of an association between measured methylation levels in blood samples and the risk of developing breast cancer was found in a large meta-analysis [15].

Following a different approach to early detect breast cancer, Beaver et al. showed that detection of circulating DNA in plasma samples identifies early-stage breast cancer patients [16]. Small, non-coding miRNAs can cause heritable changes in gene expression without altering DNA sequence similar to epigenetics. Dysregulated expression has been shown in several disease, including cancer [17]. The expression level of miRNAs were analysed in breast cancer cell lines in response to chemotherapy and showed chemotherapy-driven changes in breast cancer-related miRNAs. A pilot study with few TNBC patients proofed that previously in vitro identified miRNAs were detected in patient's urine and serum samples [18]. A prospective clinical cohort study to validate a breast cancer specific miRNA panel, suggesting that miRNA detection in urine and plasma might be used for early detection of breast cancer [19].

The fundamental weakness of all previous studies on epigenetic markers is the lack of inter-study coherence. Both in terms of the gene loci detected and the study populations,

the variance between the different studies is too big to provide a conclusive picture of potential new biomarkers.

Risk stratification

Methylation levels

Up until now, molecular classification of breast cancer has not completely ruled out a significant heterogeneity within the individual tumour subtypes. An epigenetic investigation of tumour methylation levels and patterns further refined the subtyping of carcinomas and improve personalized therapy [20,21]. Various research groups have already succeeded in developing different gene panels for epigenetic subtyping of previously diagnosed breast carcinomas. In the respective studies, these panels were usually associated with a significant difference in progression and prognosis [7,22–28], but showed little overlap between the different gene loci. However, each of these studies concluded that a high level of methylation on the panel tested was associated with poor survival, while tumours with lower methylation levels had a better prognosis. The same general pattern has been shown in an analysis of Luminal A breast cancer samples [29], suggesting a possibility to prevent overtreatment with adjuvant chemotherapy in patients with low methylation associated with a better prognosis.

Using a newly defined gene methylation panel, Stirzaker et al. further divided TNBCs into three epigenetic groups. These groups differed significantly in regards to the methylation of individual DMRs and epigenetic subtypes correlated with disease progression (high risk, intermediate risk, low risk) [30]. A recent study also demonstrated a correlation between hypermethylation of gene regions and shorter periods of remission in analysed TNBC-samples [31], providing evidence for possible clinical risk stratification and treatment planning in patients with a high risk of relapse.

Histone modifications

Another promising approach for the development of prognostic epigenetic biomarkers is the analysis of histone modifications [32]. Several studies have already suggested a correlation between aberrant expression pattern of a number of histone modifying enzymes with breast cancer prognosis [33–37]. For example, the protein arginine methyltransferase PRMT1 has been suggested to play a regulatory role in the epithelial-mesenchymal transformation (EMT) of breast cancer cells [38], in the development of cetuximab sensitivity in TNBC cell lines [39] and an association with a higher malignancy grade and unfavourable prognosis [40,41].

Interestingly, the expression of the lysine methyltransferase SETD7, has been suggested to have both a positive prognostic effect [42,43], as well as negative association with cancerogenesis and poor prognosis in patients [44,45].
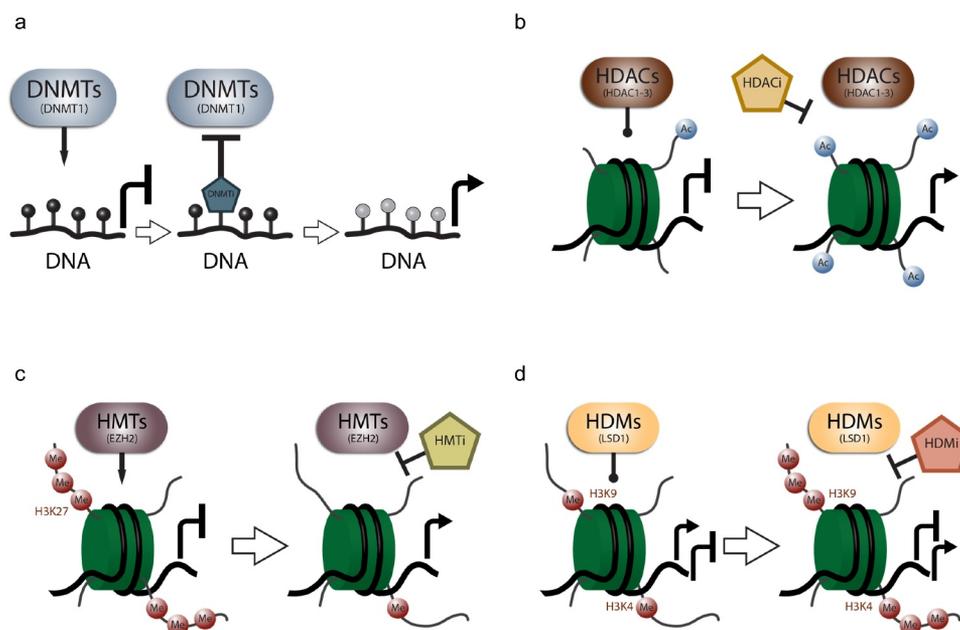
Therapy

Already established epigenetic therapies target the main players of epigenetic modification: DNA methyltransferases (DNMTs), histone deacetylases (HDACs), histone methyltransferases (HMTs) and histone demethylases (HDMs) (Figure 1). An overview of clinical trials testing epigenetic drugs in breast cancer is shown in (Table 1). (Table 2) shows selected substances including epigenetic drugs used in breast cancer.

DNMT inhibitors

The effect of DNMT inhibitors (DNMTIs) has been extensively studied alone or in combination with HDAC inhibitors (HDACIs) in breast cancer in the past decades. As early as 2006, a study showed the re-expression of oestrogen receptors after application of a combination of HDACIs and DNMTIs in TNBC cell lines [46] and a preclinical study on endocrine resistance successfully induced tamoxifen resistance in breast cancer cell lines by long-term tamoxifen application [47]. In these secondary resistant cells, a significant increase in promoter methylation of the transmembrane glycoprotein E-cadherin as well as a decrease in E-cadherin expression was detected. Application of 5-azacytidine reversed the process and re-established endocrine sensitivity to a subsequent tamoxifen therapy.

The potential reprogramming of EMT was investigated using DNMTIs and HDACIs in

Figure 1. Schematic representation of main epigenetic targets for therapy in breast cancer. (a) DNA methyltransferases (DNMTs), mainly DNMT1, induce methylation in CpG dinucleotides (black circles), which silences gene expression through recruitment of methyl-binding proteins. DNMT inhibitors (DNMTis) as decitabine and 5-azacytidine are incorporated in the DNA and block DNMT activity. Depletion of DNMTs results in demethylation of CpG dinucleotides (white circles) and de-repression of gene expression. (b) Histone deacetyltransferases (HDACs) removes acetyl groups from histone tails resulting in compacted chromatin and gene repression. HDAC inhibitors (HDACis) inhibits HDACs and resulting in histone acetylation and de-repression of gene expression. (c) The histone methyltransferase (HMT) EZH2 induces methylation of H3K27 in histone tails, which repress gene expression of target genes. HMT inhibitors (HMTis) block EZH2, decreasing H3K27 methylation and activation of gene expression. (d) The histone demethyltransferase (HDM) LSD1 has a dual effect on controlling gene expression: decreases methylation of H3K9 activating gene expression and decreases methylation of H3K4 resulting in gene repression. Depending which histone mark dominates in the nucleosome, gene expression is activated or repressed. HDM inhibitors (HDMis) block LSD1 activity reverting these histone changes.

Table 1. Overview of selected clinical studies on epigenetically active substances. *: no official evaluation available yet. Study was stopped prematurely due to lack of significant survival benefit of combination therapy. ORR: overall response rate. pCR: pathological complete response; TNBC, triple negative breast cancer; ER, oestrogen receptor; PR, progesterone receptor; UICC, Union for International Cancer Control.

Substance(s)	Phase	Patients #	Patient characteristics	ORR	Reference
5-azacytidine + Entinostat	II	40	TNBC (n = 13), hormone resistant (n = 27)	4% (hormone resistant); 0% (TNBC)	Connolly et al., 2017 [55]
Vorinostat	II	14	UICC IV (ER/PR+ (n = 8), Her2+ (n = 4)	4/14 (28%) stable disease	Luu et al., 2008 [64]
Vorinostat + Paclitaxel + Trastuzumab	I+ II	55	UICC IIa-IIIc (Her2+ (n = 26), TNBC (n = 16), ER/PR+ (n = 13)	pCR: 54%(Her2+); 27% (TNBC); 0% (ER/PR+)	Tu et al., 2014 [65]
Vorinostat + Ixabepilone	Ib	49	UICC IV (TNBC (n = 15))	22% (every 3 weeks); 30% (weekly)	Luu et al., 2018 [66]
Vorinostat + Tamoxifen	II	43	UICC IV: ER+ with tumour progression under hormone therapy	19%	Munster et al., 2011 [7374]
Vorinostat + Pembrolizumab + Tamoxifen	II	ongoing	UICC IV: ER+	ongoing	University of California, San Franciso
Entinostat + Exemestane	III	*	ER+ with tumour progression under hormone therapy	*, no overall survial benefit	Yeruva et al., 2018 [70]

Table 2. Overview of selected epigenetic drugs, chemotherapies, hormone and targeted therapies used in breast cancer and their proposed molecular function. Several of the already approved agents in other solid and haematological malignancies are currently being tested in clinical trials to expand clinical approval in breast cancer. AML, acute myeloid leukaemia; MDS, myelodysplastic syndrome, CMML, chronic myelomonocytic leukaemia; JMML, juvenile myelomonocytic leukaemia; CTCL, cutaneous T-cell lymphoma; NSCLC, non-small cell lung cancer; PMF, primary myelofibrosis; ER, oestrogen receptor; SERM, selective oestrogen-receptor modulator; FDA, United States Food and Drug Administration.

Drug	Molecular function	Phase	Disease
DNMTIs			
5-azacytidine	cytidine analog: demethylating agent at low dose, direct cytotoxicity at high dose	approved	AML, MDS, CMML, JMML
Decitabine	cytidine analog: demethylating agent at low dose	approved	AML, MDS
Guadecitabine	cytidine analog: demethylation agent at low dose, can not be degraded by cytidine-deaminase	Phase III	haematological diseases, solid cancers
HDACIs			
Vorinostat	inhibitor of histone classes I, II, IV	approved (only FDA)	CTCL
Entinostat	inhibitor of HDAC1, HDAC3	phase III	various cancers
Chemotherapy agents			
Paclitaxel	taxane: disruption of microtubule function	approved	several solid cancers
Ixabepilon	disruption of microtubule function	approved (only FDA)	breast cancer second line
AFP464	cytotoxic pro-drug: aminoflavone	phase II	solid cancers
Cisplatin	DNA-crosslinking interfering with cell division by mitosis.	approved	several solid cancers
Capecitabine	Prodrug of 5-fluoruracil, antimmetabolite	approved	breast colon and gastric cancer
Hormone therapy			
Exemestan	aromatase inhibitor	approved	post-menopausal ER+ breast cancer
Tamoxifen	selective oestrogen-receptor modulator (SERM)	approved	pre-menopausal ER+ breast cancer
Immunotherapy			
Trastuzumab	monoclonal antibody targeting HER2	approved	HER2+ breast cancer
Pembrolizumab	PD-1 antibody	approved	NSCLC, metastatic melanoma
Cetuximab	Monoclonal antibody targeting epidermal growth factor receptor (EGFR)	approved	Head and neck cancer, colon cancer
Targeted therapy			
Ruxolitinib	JAK1/JAK2 inhibitor	approved	PMF

human TNBC cells [48]. As previously mentioned, this central part of the metastatic process of malignant human cells appears to be epigenetically regulated, at least in part by promoter hypermethylation and HDAC-mediated regulation of associated repressors [49,50]. Su et al. investigated the possibility of converting highly aggressive TNBC cells post-EMT into a less aggressive state by means of epigenetic drugs: the combination of the second-generation DNMTI guadecitabine with the HDACI entinostat showed the greatest antitumor effect in patient-derived xenograft mouse models [48].

In addition, it has been shown in various breast cancer mouse models that guadecitabine treatment can target the pathologically increased myelopoesis in the bone marrow [51]. The associated decrease in myeloid-derived suppressor cells (MDSCs) increased the efficacy of co-applied immunotherapy and consequently reduced the tumour burden.

In early clinical trials, the established DNMTIs 5-azacytidine and decitabine did not show a major therapeutic effect when administered as monotherapy [52] but preclinical and clinical studies of different cancer entities showed evidence of a synergistic effect in combination with HDACIs [53,54]. However, a phase II clinical trial of the combination of 5-azacytidine and entinostat in hormone-refractory breast cancer patients did not confirm this hypothesis [55] (Table 1). Due to the heterogeneity of the disease, it seems likely that only specific breast cancer subgroups will benefit from these therapies. Approaches to identifying these patients should be further investigated. Initial studies have identified DNMT expression in TNBCs

patient derived-xenograft mouse models as a first potential marker for the response to decitabine [56].

Interestingly, combination of DNMTIs with PARP-Inhibitors (PARPIs) enhances the cytotoxic effect of PARP-inhibition in human BRCA-wild type TNBC cell lines due to a mechanistic relationship between these two agents that increased the amount of PARP1 at locations of DNA damage [57]. Homologous recombination deficiency (HRD) is one central characteristic of BRCA-mutant cancer cells and confers sensitivity to PARP-inhibition. Recently, it has been shown that DNMTIs might also be able to induce a BRCA-mutant state in BRCA-wild type TNBC cells by promoting HRD [58]. These very recent findings serve as the backbone of a now-enrolling clinical trial in breast cancer patients with wild-type BRCA with the intention of broadening the scope of PARPi therapy in breast cancer.

HDAC inhibitors

HDAC inhibitors (HDACIs) can be divided into five different classes: (I) hydroxamic acids, (pan-HDACIs, i.e., vorinostat) (II) short chain fatty acids (class I and II HDACIs, i.e., valproate), (III) benzamides (specific class I HDACIs, i.e., entinostat), (IV) cyclic tetrapeptides (specific class I HDACIs, i.e., romidepsin) and (V) sirtuin inhibitors (pan-HDACIs including SIRT1 and SIRT2, i.e., nicotinamide) [59].

In the recent past, preclinical studies have primarily focused on the possibilities of combination therapies. AFP464 is a pro-drug whose efficacy is currently being investigated in a phase II clinical trial in ER+ breast cancer with progression under AI-therapy [60]. In a TNBC-xenograft mouse model, a combination of AFP464 and vorinostat has been shown to increase AFP464 sensitivity in a vorinostat-associated manner. At the same time, the co-application of vorinostat potentiated the anti-tumoural activity of AFP464 [61] (Figure 1). The combination of vorinostat with cisplatin also showed an additive therapeutic effect when compared to cisplatin monotherapy in various breast cancer cell lines [62] (Figure 1). In addition, it was shown that HDACIs must be given before the application of other anti-tumour agents in order to trigger an increase in cytotoxicity at least in human breast cancer cell lines. Therefore, it has been hypothesized that HDACIs such as vorinostat mediate their priming effect by loosening the chromatin structure and thus improving accessibility to subsequently administered substances [63] (Figure 2).

A first small phase II study in patients with metastatic breast cancer indicated that the therapeutic benefit of vorinostat was observed primarily in combination with established therapeutic strategies [64]. In a phase I/II study published in 2014 on the possibility of combining vorinostat with paclitaxel in locally advanced breast cancer patients (stage IIA-IIIC), a high rate of complete response with tolerable toxicity was achieved in the Her2+ subgroup when trastuzumab was added [65]. In 2018, the results of a phase Ib study were published, which investigated the combination of the cytostatic drug Ixabepilon with vorinostat in stage IV breast cancer patients (Table 1). The objective response rates were comparable to those achieved with the previously approved Ixabepilon monotherapy or combination with capecitabine. Most promising, however, was the fact that this therapeutic success was accompanied by a reduction of side effects [66].

HDAC inhibitors as therapy sensitizers

One particular benefit of integrating HDAC inhibitors into therapy regimens could be their potential to sensitize malignant cells to subsequent anti-tumourous agents applied later in the therapeutic cycle.

One therapeutic approach for patients with ER breast cancer is the induction of oestrogen receptor expression and the associated development of hormone sensitivity. In this context, preclinical studies identified the potential of the HDACI entinostat. The application of entinostat has shown a re-expression of oestrogen receptors both in vitro and in xenograft models of previously ER-negative breast cancer. As a consequence, the formerly resistant cells became (re)sensitive for a subsequent endocrine therapy [55,67,68].

Based on the success of a large phase II study (Encore301) in patients with ER+ breast cancer
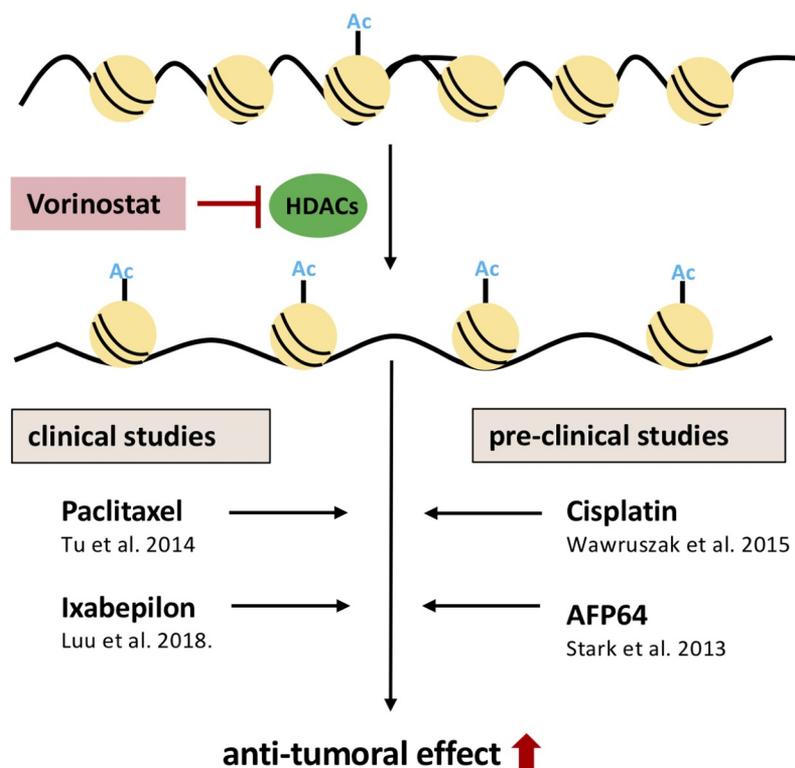
Figure 2. Vorinostat increases the effect of established anti-tumour substances by opening chromatin. The loosening of the chromatin structure mediated by Vorinostat increases the accessibility for later applied substances (Kim et al. 2003). Pre-clinical as well as clinical studies have shown an associated increase in the anti-tumoural effect of various chemotherapeutic substances.

with progression under hormone therapy [69], an international phase III study (E2112) was conducted to combine the aromatase inhibitor exemestane with entinostat [70]. However, in May 2020 the responsible pharmaceutical company announced that the study failed to demonstrate a statistically significant overall survival benefit of the combination therapy compared to hormone therapy alone [71].

Changes in epigenetic signalling pathways triggered by endocrine therapies might induce secondary therapy resistance. The subsequent alteration of the transcriptome might enable the development of an endocrine-refractory phenotype. Epigenetic reprogramming of these altered signalling pathways could thus represent a promising therapeutic approach as shown in breast cancer cell lines [72]. In this context, a phase II study was conducted to test the combination of vorinostat and tamoxifen in patients with hormone-refractory breast cancer who had previously experienced progression under hormone therapy [73]. The well-tolerated combination therapy led to an objective response rate in 19% of cases and produced a clinical benefit in 40% of patients. In addition, a phase II study has just started to evaluate the role of vorinostat as a potential epigenetic 'primer' in combination therapy with pembrolizumab and tamoxifen in ER+ metastatic breast cancer [74] (Table 1).

Solid tumours tend to develop rapid resistance to HDACI monotherapy [75]. A 2016 publication provides a promising explanation: In addition to the intended anti-tumour effect, HDAC inhibition seems to have a parallel anti-apoptotic, JAK-mediated pathway, which undermines the primary therapeutic target. Based on this potential signalling pathway, a combination of HDACIs and JAK/BRD4-inhibitors showed a significantly increased sensitivity of TNBC cells to HDAC inhibition [76]. As there already is a pan-JAK1/2 inhibitor (Ruxolitinib) in clinical use for treatment of myelofibrosis [77], this combination could be a promising therapeutic approach to make already established epigenetic therapies accessible to solid tumours.

HMT inhibitors

The Enhancer of Zeste Homolog 2 (EZH2) is a histone methyltransferase, which belongs to the Polycomb Repressive Complex 2 (PRC2) enzymatic complex. It undergoes post-translational modifications as phosphorylation by the kinase p38 [78]. Phosphorylated pEZH2 is able to re-localize into the cytoplasm and activate pro-metastatic signalling pathways in human clinical samples of invasive breast cancer and metastasis. Thus, targeted inhibition of EZH2 and p38 could be a potential strategy for the therapy of difficult-to-treat ER+ breast cancer, especially tumours with high pEZH2 expression, to prevent or interrupt distant metastasis [79].

A paper published in 2018 identified a new building block of the EZH2 signalling pathway with potential implications for endocrine breast cancer therapy [72]: GREB1, a co-factor of the oestrogen receptor alpha (ERα) can be transcriptionally inhibited by EZH2-mediated methylation. The associated decrease in GREB1 expression leads to a dysfunction of ERα, which in turn results in a decrease in cellular hormone sensitivity. A review of the signalling pathways in the tumour material of patients with ER+ breast cancer has shown an association between tamoxifen resistance and a higher level of EZH2. This increase in EZH2 also showed an association with poorer disease-free survival. Furthermore, the authors derived prognostic factors from their work: a high EZH2 expression and low GREB1-expression correlated with a negative clinical course (Figure 3).

PARP inhibitors have been tested in clinical trials in BRCA-mutant breast cancer [80] and received approval by the FDA and EMA for the therapy in metastatic disease [81–84]. However, the mechanisms that make cells sensitive to PARP inhibition are still largely unclear. In 2018, Yamaguchi et al. demonstrated an association between PARP and EZH2 [85]: Triggered by oxidative stress or DNA damage, a PARP-mediated structural modification of EZH2 reduces its methylation activity in BRCA-mutated breast cancer cell lines. This PARP-mediated decrease in EZH2 activity appears to lead to a decrease in methylation-associated gene repression and thus to a decrease in the oncogenic function of EZH2. Thus, in addition to the intended effect, therapeutic PARP inhibition might reactivate the oncogenic effect of EZH2 and thereby lead to therapy resistance. Based on these results, Yamaguchi et al. combined PARP inhibition and EZH2 inhibition. Both in vitro and in vivo, this combination showed
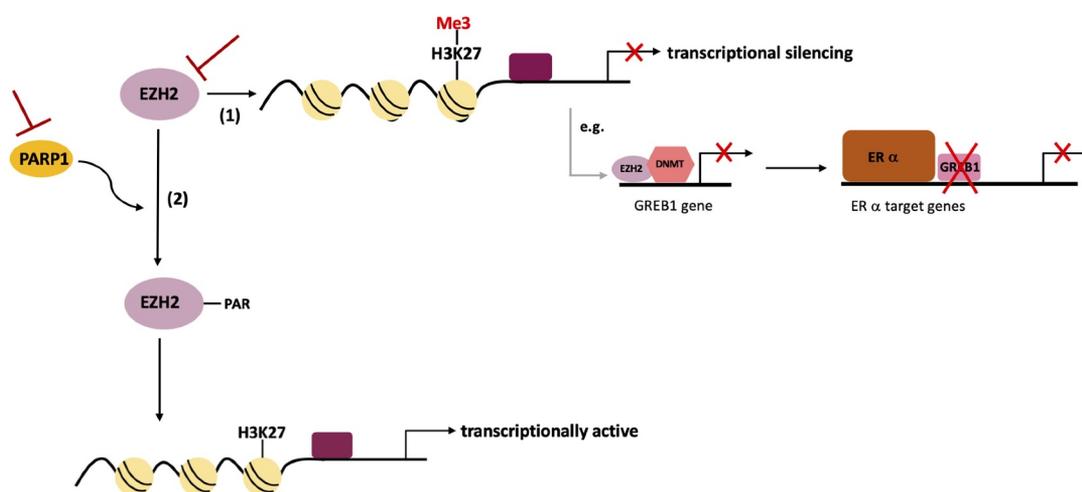
Figure 3. EZH2 plays a crucial role in various epigenetic signalling pathways of breast cancer. (1) EZH2-mediated histone methylation, in cooperation with DNMTs, inhibits the function of the oestrogen receptor alpha by transcriptional silencing of the co-factor GREB1, which in turn leads to a decrease in cellular sensitivity to endocrine substances (Wu et al. 2018). (2) EZH2 modification by PARP1, triggered by cellular stress, reduces its methylation capacity and thus its oncogenic function. PARP inhibition can reverse this process and thus reactivate the oncogenic function of EZH2 in contrast to the primary therapeutic goal (Yamaguchi et al. 2018). Targeted EZH2 inhibition could intervene in both signalling pathways and thus represents a potential therapeutic approach.

evidence of increased sensitivity to PARP inhibition. Thus, targeted EZH2 inhibition seems to be promising as monotherapy for (secondary) tamoxifen resistance and in the context of combination therapies to increase sensitivity to established therapies (Figure 3).

HDM inhibitors

Histone demethylases (HDMs) catalyse the demethylation of lysine and arginine residues on histone tails. Due to their central role in the maintenance of cancer stem cells (CSCs) [86–88], they also offer therapeutic potential. In 2010, an association between increased expression of lysine-specific demethylase 1 (LSD1) and ER-negative breast cancer was shown. However, an increase in LSD1 expression is not specific for breast cancer. Rather, it seems to be a fundamental characteristic of highly aggressive tumour biology [35].

Current pre-clinical studies are investigating the therapeutic potential of LSD1 inhibition. In TNBC samples, LSD1 inhibition reduced CSC levels and in the derived TNBC-xenograft mouse model, LSD1 knockout led to slower tumour progression [87]. Overexpression of the transcription factor SOX2 is necessary to maintain the CSC phenotype mammosphere formation of breast cancer cell lines. In 2020, a functional SOX2-LSD1 axis was demonstrated in which LSD1 can trigger an increase in SOX2 expression via enhancer activation. Based on these findings, LSD1 inhibition could reduce colony formation and a decrease in SOX2 expression, especially in multi-drug-resistant human luminal B tumours [86]. Further studies showed a reduced stem cell potential of the examined CSCs after LSD1 inhibition, as well as an increased chemo-sensitivity of human breast cancer cell lines when combined with classical chemotherapeutic agents such as doxorubicin [88].

LSD1 inhibition in combination with anti-PD1 antibodies showed an anti-tumoural effect in a TNBC mouse model with previously established immunotherapy resistance [89]. In a newly developed xenograft model, Metzger et al. also demonstrated reduced tumour growth of patient-derived CSCs after LSD1 inhibition

[90]. In addition, the application of a dual HDM inhibitor (MC3324) in cell culture showed the same hormone modulatory effects as established hormone therapy [91].

The promise of combination therapy

As previously pointed out, epigenetic monotherapy has produced limited results in several solid tumour entities [52,64,75], causing an increased focus on the potential of combination therapy. One central premise is based on the hypothesis that aberrant epigenetic signalling plays a central role in the development of inherently therapy resistant cancer stem cells. Targeting epigenetic mechanisms within the cancer cell would therefore challenge the 'stemcellness' of those therapy resistant cells, the very characteristics that render them resistant to established therapy regimes in the first place [92–94]. However, combination therapies can only be implemented successfully if they are based on an informed choice formed on extensive studies on the mechanistic nature of both the applied drugs and the targeted pathways [95,96].

Adverse effects of epigenetic therapy

The general challenge of established epigenetic drugs is their target unspecificity. The currently available epigenetic drugs appear to have a global effect on the epigenome rather than on specific aberrant targets [97–99], causing a global hypomethylation with a potentially harmful impact on the formation of cancer [100]. Dose-specific mechanisms should be considered as well when planning combination therapies with minimal adverse effects. At a low dose, both 5-azacytidine and decitabine can inhibit the clonogenicity of the tumour cell lines and primary tumour tissues via demethylation-mediated epigenetic reprogramming. Cytotoxicity appears to be avoided at lower doses, and bone marrow progenitors are spared, likely because they function independent of aberrant hypermethylation. At higher doses, both drugs can cause unwanted direct cytotoxicity, resulting in an increase of side effects [101],

Conclusion and outlook

Regarding the development of epigenetic biomarkers for early diagnosis and subsequent risk stratification, the biggest hurdle for the clinical transfer is the current lack of large prospective studies with adequate validation. Should this research gap be closed in the future, an epigenetic advancement of current practice seems possible and promising, especially in regard to the development of miRNA-based biomarkers.

Regarding the future of epigenetic therapies, a more rational approach to planning and designing combination therapies, both pre-clinical and clinical, has to be adapted. So far, the developed epigenetic substances did not live up to their expected effects when applied as monotherapy, even though previous mechanistic studies have suggested a crucial role in cancerogenesis. Therefore, the biggest rational potential for clinical success of epigenetic agents appears to be the integration into combination therapy regimens, especially regarding both primary therapy resistance and the development of secondary resistance. Combining epigenetic agents with established PARP-inhibitors is a particularly promising approach in this matter.

In conclusion, several aspects of epigenetic research show significant potential for clinical application but the focus on a rational mechanism-based approach and increased interdisciplinary research is of utmost importance for the future success of this field. Doing so could help pre-clinical advances reach their full clinical potential and improve both current diagnosis and therapy strategies of breast cancer, especially in difficult to treat entities like TNBC and metastatic disease.

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ORCID

Jesús Duque-Afonso http://orcid.org/0000-0002-8287-5673

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