Review

Pharmacogenomics

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The pharmacoepigenetics of drug metabolism and transport in breast cancer: review of the literature and *in silico* analysis

The focus of this manuscript is on DNA methylation and miRNA regulation of drug-metabolizing enzymes and drug transporters involved in the disposition of drugs commonly used in breast cancer. We start with a review of the available scant literature and follow with an *in silico* analysis of the CpG islands and miRNA binding sites of genes of interest. We make the case that there is room for further research to include more genes and miRNAs despite the extensive sharing of miRNA targets by candidate genes of interest. We also stress on the role of peripheral blood as a source of pharmacoepigenetic biomarkers, and point out the lack of toxicoepigenetic studies in breast cancer.

First draft submitted: 5 May 2016; Accepted for publication: 1 July 2016; Published online: 26 August 2016

Keywords: breast cancer • drug-metabolizing enzymes • drug transporters • epigenetics • pharmacoepigenetics • pharmacogenetics • toxicoepigenetics

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Breast cancer

Breast cancer is a major public health problem that, worldwide, affects around one million women every year [1]. Despite advances in detection and treatment methods, breast cancer still remains the number one cancer killer of women worldwide. Genetic factors are thought to represent important determinants of efficacy and toxicity of treatment. For instance genetic polymorphisms in drug-metabolizing enzymes (DMEs) involved in metabolism and drug transporters involved in drug absorption, uptake and efflux from target cells, and elimination have been implicated in toxicity and inappropriate response from standard doses of breast cancer therapy [2–6].

Drug metabolism & transport

The expression and function of both DMEs and transporters are critical to drug disposition and therapeutic outcome. Drug metabolism is a major route of elimination from the body for most drugs. It is mainly mediated by two classes of DMEs: Phase I oxidation enzymes, the most important of which is the CYP system, and Phase II enzymes which include a variety of conjugation enzymes such as Glutathione *S*-transferases (GSTs). CYPs have different substrate selectivity and are classified into several families and subfamilies, and the most clinically relevant enzymes involved in metabolizing 90% of US FDA approved drugs are CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2B6, CYP2D6, CYP2E1 and CYP3A [7]. DMEs are mainly expressed in the liver, yet they have also been shown to be expressed in a multitude of other tissues including placenta and tumor tissue such as breast cancer tissue [8,9].

Drug transporters are essential players for drug uptake and excretion, and are divided into two main families: the ABC family that is mainly involved in drug efflux and includes the MDR P-glycoproteins; and the SLC family that is mainly involved in drug uptake. The role of drug transporters is to facilitate the passage of drugs through the

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cellular cytoplasmic membrane from, for example, the intestine into blood and from blood into the kidneys' tubular lumen [10]. ABC transporters are the largest transmembrane protein family, and this family includes 48 identified members, out of which ten are implicated in drug resistance through hindering the drug's ability to reach its site of action. P-glycoprotein is a product of *MDR1* also known as *ABCB1* which is overly expressed in breast cancer. Other members such as ABCG2 also known as breast cancer resistance protein were found to be implicated in chemotherapeutic drug resistance [11].

Pharmacoepigenetics

Several pharmacogenetics studies have attempted to identify genetic polymorphisms in genes encoding these DMEs and transporters accounting for the interindividual variation with drugs including cytotoxic drugs in breast cancer; yet results were many times conflicting and nonconclusive. For example, some reported an increase in docetaxel clearance in carriers of *CYP3A4* polymorphisms [12,13], a finding that was not duplicated by others [14]. Similar less clear data were shown with the most extensively studied *ABCB1* candidate gene. For instance, although the C1236T SNP was shown to be associated with lower clearance of docetaxel [14], no significant relationship was found with docetaxel toxicity [15]. This is in contrast to the C3435T and G2677T/A variants that, although they were not previously shown to affect docetaxel kinetics, they were associated with neutropenia [12–15]. These conflicting results may be attributed to differences in breast cancer molecular subtypes, chemotherapy regimen and toxicity end points among the studies. In addition, alternative changes in gene expression that are not related to genetic polymorphisms offer another potential mechanism for explaining interindividual variability in disease treatment response and toxicity. These changes are collectively described as epigenetics, with pharmacoepigenetics and toxicoepigenetics being the application of epigenetics to pharmacology and toxicology, respectively [16–18].

Pharmacoepigenetics of drug metabolism & transport in breast cancer: literature review

Epigenetics plays an important role in regulating gene expression. It is a bridge between environmental and genetic factors. It includes DNA methylation, miRNA expression and histone modifications [19]. There is emerging evidence on the role of epigenetic biomarkers in breast cancer susceptibility and treatment. The focus of this review is however on DNA methylation and miRNA regulation of DMEs and drug transporters involved in the disposition of drugs commonly used in breast cancer. The reader is referred to selected recent reviews for additional genes and pathways of interest in breast cancer such as estrogen and estrogen receptors, apoptosis, cell division and invasion, DNA transcription, signal transduction and DNA repair including *BRCA* [20–25]. There are also some recent reviews on pharmacoepigenetics in general [16,26,27] and others with a focus on drug metabolism and transport [17,28,29]. Most of the available literature is on ABC transporters that export the drug out of the cell and few candidate DMEs.

DNA methylation

The addition of methyl moieties to cytosine at CpG sites generates methylcytosines that can either block the binding of transcription factors or attract methylated – CpG-binding proteins and other chromatin-remodeling enzymes that create a closed chromatin configuration. This DNA methylation and/or altered chromatin configuration prevents gene expression, hence affecting many cellular and physiologic processes [29]. High concentrations of CpG islands are most often found at promoter regions and first exons, but they may also occur in regions toward the 3′-end. The CpG islands are normally unmethylated in transcriptionally active genes like housekeeping and DNA repair genes, whereas developmental and tissue-specific genes mostly appear to be methylated and silenced in differential tissues [29].

DNA methylation has been most studied in assessment of cancers' signatures whereby numerous genes which are unmethylated in the nonmalignant tissue become aberrantly methylated in the tumor [25]. In breast cancer tissue, global DNA hypomethylation is apparent when compared with normal tissue counterparts [30,31]. Though a bit rare, DNA hypomethylation can affect individual genes, and in breast cancer cell lines and tissues, this is the case for few genes including *ABCB1* [32,33]. Hypermethylation can also occur, and in breast cancer the DME *GSTP1* gene was found to be so [34].

To our knowledge, there are six studies that evaluated the role of DNA methylation in DMEs and or drug transporters in breast cancer (Table 1). A couple of studies focused on the role of DNA methylation in the resistance of breast cancer cell lines to chemotherapeutic agents and in poor treatment outcome. For example, hypomethylation of *MDR1* gene was observed in the multidrug resistant human breast cancer MCF-7 cells (MCF-7/ADR) that express high *MDR1* mRNA levels when compared with the breast cancer MCF-7 cells. Chromatin immunoprecipitation revealed the presence of active *MDR1* chromatin conformation, DNA methylation binding proteins MeCP2 and MBD2,

Table 1. Summary of the literature on consequences of methylation and or miRNA interaction of drug-metabolizing enzymes and transporters on drug response in breast cancer.

Drug	Target DME or transporter	Epigenetic mechanism	Breast tissue or cell line	Consequence of low miRNA expression or hypomethylation	Ref.
Multiple drugs	ABCB1 (MDR1)	Methylation	MCF-7/ADR	Multidrug resistance	[35]
Doxorubicin	ABCB1 (MDR1), GSTπ, MGMT	Methylation	MCF7/DOX	Drug resistance	[36]
	ABCB1 (MDR1), GSTP1	Methylation	Breast cancer tissues	Poor survival	[37]
	ABCB1 (MDR1)	miRNA-451	MCF-7/DOX	Drug resistance	[38]
	ABCB1 (MDR1)	miRNA-298	MDA-MB-231	Drug resistance	[39]
	ABCC5	miRNA-128	Breast tumor initiating cells	Drug resistance	[40]
Etoposide	ABCC1 (MRP1)	miRNA-326	MCF-7/VP	Drug resistance	[41]
Cisplatin	ABCC1 (MRP1)	miRNA-7 miRNA-345	MCF-7/CDDP	Drug resistance	[42]
Methotrexate	ABCG2 (BCRP)	miRNA-328 miRNA-487a	MCF-7/MX100	Drug resistance	[43,44]
Tamoxifen	NAT1	Methylation	Cancer tissues	Drug sensitivity	[45]
	PSAT1	Methylation	Cancer tissues	Poor clinical outcome	[46]
	CYP1B1	Methylation	Cancer tissues	Poor survival	[47]
Estrogen	CYP1B1	miRNA-27b	MCF-7	NA	[48]

DME: Drug-metabolizing enzyme; MDR: Multidrug resistance; NA: Not applicable.

transcriptional trans-activator YB-1 and significant amounts of acetylated histones in MCF-7/ADR cells while there was inactive *MDR1* chromatin conformation and few acetylated histones in MCF-7 breast cancer cells [35]. In addition, doxorubicin resistant MCF-7 variant (MCF-7/DOX) was established by stepwise selection of MCF-7 cells treated with doxorubicin, and the epigenetic and gene expression changes associated with resistance to doxorubicin were studied. Results showed hypomethylation of *MDR1*, *GSTπ*, *MGMT*, and *Upa* genes in MCF-7/DOX when compared with the MCF-7 cell line. Interestingly and as expected, the decrease in *MDR1* methylation was correlated with an increase in *MDR1* expression [36].

Few other studies identified DNA methylation markers that predict response to cytotoxic and hormonal therapy in breast cancer tissues of patients. For instance, pyrosequencing was performed to evaluate DNA methylation of promoters in 14 genes in tissues derived from a discovery cohort of 75 patients with locally advanced breast cancer assigned for primary treatment with doxorubicin, and a validation cohort of 163 breast cancer patients. Selection of genes was based on previously reported DNA methylation in breast cancer cells or in other cancer cells (*ABCB1*, *ATM*, *BRCA1*, *CDH3*, *CDKN2A*, *ESR1*, *GSTP1*, *IGF2*, *HMLH1*, *PPP2R2B*, *PTEN* and *CXCR4*), differential gene expression in breast cancer cells (*FOXC1*) or tumor suppression activity (*FBXW7*). Of all genes analyzed, methylated *FOXC1* was found to be associated with survival in both discovery and validation cohorts when compared with unmethylated *FOXC1*, so it was considered as a general prognostic factor. Unmethylated *ABCB1* and *GSTP1* were associated with poorer survival in the discovery cohort only when compared with methylated *ABCB1* and *GSTP1*, hence *ABCB1* and *GSTP1* methylation may be considered predictors of doxorubicin response in breast cancer patients [37].

As for studies on tissues with endocrine therapy such as tamoxifen, Kim *et al*. evaluated the association of DNA methylation and RNA expression of genes encoding five DMEs, commonly expressed in breast tissues, including *COMT*, *CYP2D6*, *NAT1*, *CYP1A1* and *SULT1A1* with tamoxifen resistance in breast cancer patients. Bisulfite sequencing and methylation specific PCR (MSP) were used to measure the methylation levels of the five genes in tamoxifen-sensitive (n = 74) and tamoxifen-resistant (n = 37) breast cancer tissues. Of the five genes studied, only *NAT1* showed a higher methylation frequency in tamoxifen-resistant patients (62.2%) than in tamoxifen-sensitive patients (33.8%), and this was associated with lower *NAT1* expression in tamoxifen-resistant cases than in the control group [45]. Besides, microarray-based DNA methylation analysis of 117 candidate genes was performed in tumor tissue samples derived from a cohort of 200 patients receiving tamoxifen alone as a first-line treatment for recurrent breast cancer. The DNA methylation of ten genes was associated with progression-free survival after initiation of treatment with tamoxifen. Among these ten genes, the hypermethylation status of *PSAT1* was most significantly associated with favorable tamoxifen clinical outcome. Moreover, an inverse relationship was reported between hypermethylation and mRNA levels of *PSAT1*, and both were reported as predictors of favorable response to therapy [46]. In addition, Widschwendter *et al.* selected and measured the methylation of 35 DNA methylation markers on the basis of previous reports in tumor tissues derived from 148 patients with primary breast carcinomas using the MethyLight assay. Results showed that breast cancer patients with high *ERa* DNA methylation had better survival with tamoxifen than patients with low *ERa* DNA methylation. Similarly, high *ARH1* methylation was associated with better response to tamoxifen. Furthermore, DNA methylation of *CYP1B1* predicted response differentially in tamoxifen and nontamoxifen-treated patients whereby tamoxifen-treated patients with high DNA methylation of *CYP1B1* showed better survival than tamoxifen-treated patients with low DNA methylation of *CYP1B1*. Conversely, nontamoxifen-treated patients with high DNA methylation of *CYP1B1* had worse survival when compared with those with low *CYP1B1* DNA methylation. As discussed by the authors, the involvement of CYP1B1 in the metabolism of tamoxifen/estradiol may explain the differential effect of *CYP1B1* DNA methylation in tamoxifen and nontamoxifen groups of patients. High DNA methylation of *CYP1B1*, if associated with less *CYP1B1* expression, would result in lower rates of tamoxifen metabolism to inactive metabolites, leading to accumulation of the antiestrogen and better clinical response to tamoxifen. However, in nontamoxifen-treated patients, the decrease in *CYP1B1* expression would result in less estrogen metabolism to its weaker catechol derivatives and eventually worse clinical response [47].

miRNA expression

miRNAs are short noncoding sequences of RNA ranging from 19 to 22 nucleotides. They are endogenous molecules of important regulatory effects at the level of post-transcription, whereby they inhibit mRNA translation or allow its degradation by binding to its 3′-UTR [49,50]. Although these miRNAs represent about 1% of the genetic material, it is estimated that they are involved in the regulation of about 30% of the human genome [51]. Certain families of miRNAs were found to be dysregulated in breast cancer, acting as oncogenes or tumor suppressor genes and having significant impact on cancer progression and drug resistance. Recent large-scale profiling approaches have revealed that miRNAs are mostly globally downregulated in several cancer types including breast cancer [52,53]. Since miRNAs were found to classify human disease with better precision than gene expression [54], it is expected that they will also superiorly categorize drug response [22,55].

To our knowledge, most of the miRNAs-mediated drug disposition research involved miRNA regulation of ABC transporters (Table 1). Drug efflux through certain pumps is one of the main reasons of drug resistance. Certain miRNAs affect the expression of these efflux proteins and ultimately disrupt the sensitivity of cancer cells to chemotherapeutic agents. For example, doxorubicin resistance was tested using doxorubicin MCF-7 resistant cells whereby high protein levels of P-glycoprotein were expressed in MCF-7/DOX cells compared with MCF-7 cells. It was found that transfecting resistant cells with miRNA-451 caused a decrease in the levels of expression of MDR1 compared with MCF-7 cells. Furthermore, a 2.5-fold lower IC_{50} of doxorubicin was estimated in treated MCF-7/DOX after transfection with miRNA-451 when compared with treated MCF-7/DOX cells transfected with a control vector [38]. Similar results were found with miRNA-298 and doxorubicin in MDA-MB-231. For instance, doxorubicin was found to be localized more in the cytoplasm when compared with the nucleus of MDA-MB-231 resistant cells leading to suboptimal effect. This was explained by the presence of higher levels of MDR1 at the nuclear level in MDA-MB-231 resistance cells; accordingly inhibiting these efflux pumps with verapamil had shown improvement in doxorubicin nuclear translocation and hence greater cytotoxic effect. MiRNA assay showed a significant lower expression of miRNA-298 levels in MDA-MB-231 resistant to doxorubicin when compared with sensitive MDA-MB-231 cells. Moreover, Dicer was found to be downregulated in resistant cells leading to lower expression of mature miRNA levels. Interestingly, overexpressing miRNA-298 in MDA-MB-231 resistant cells enhanced doxorubicin cytotoxic effect by increasing its concentration at the level of nucleus, and this was associated with decreased levels of MDR1. Similar results were shown with MCF-7/DOX cells [39]. More recently, it was found that breast tumor-initiating cells had reduced miR-128, and that ectopic expression of miR-128 was associated with a decreased expression of ABCC5 and proto-oncogene polycomb ring finger (Bmi-1) and a better response to doxorubicin [40].

Resistance against etoposide (VP-16), a cytotoxic topoisomerase inhibitor, is also of importance and this is partly attributed to the higher MRP1 (ABCC1) levels in MCF-7/VP cells compared with MCF-7 cells. Out of many miRNA levels identified using q-RT PCR, miRNA-326 levels were found to be 3.3-fold less expressed in MCF-7/VP cells compared with MCF-7 cells, and this was also the case in advanced breast cancer tissues compared with normal breast tissue. It was shown that MRP-1 levels decreased in MCF-7/VP cells transfected with miRNA-326 compared with control, and that there was a decrease in IC_{50} of VP-16 by 7.1-fold in MCF-7/VP cells transfected with miRNA-326 when compared with those transfected with empty vector [41]. Another cytotoxic drug that was studied is cisplatin, whereby it was confirmed that higher levels of MRP1 (ABCC1) were present in MCF-7 cells resistant to Cisplatin (MCF-7/CDDP cells) when compared with MCF-7 cells, and this was associated with lower levels of miRNA-7 and miRNA-345 in the resistant cells. As expected, transfection of MCF-7/CDDP cells with miRNA-7 and miRNA-345 expression vectors was associated with a decrease in IC_{50} of Cisplatin when compared with MCF-7/CDDP cells transfected with empty vector [42].

ABCG2 (breast cancer resistance protein) is also one of these efflux pumps that may affect the pharmacokinetics (PKs) of chemotherapeutic agents and eventually their ability to produce effect. In MCF-7/MX100 cells that are resistant to methotrexate, it was found that expression of ABCG2 was sixfold higher when compared with MCF-7 cells. This was associated with miRNA-328 levels being significantly lower in MCF-7/MX100 cells as compared with MCF-7 cells, and transfection of the resistant cells with miRNA-328 plasmid led to a 70% decrease in the levels of ABCG2 when compared with MCF-7/MX100 cells transfected with normal vector. Moreover, transfection with miRNA-328 was associated with a decrease in methotrexate IC_{50} [43]. Therefore in breast cancer, methotrexate resistance can be partly due to miRNA-328 downregulation that causes an upregulation of ABCG2 and eventually less methotrexate reaching therapeutic targets in the nuclei of cancer cells. Similar results were found with miRNA-487a and methotrexate resistance in MCF-7/MX100 cells, and this was further confirmed using a human MCF-7/MX-bearing nude mice xenograft mode [44]. In conclusion, the negative regulation that links the expression of certain miRNAs with their target drug efflux proteins may explain the resistance of certain breast cancer cell lines or tissues to chemotherapeutic drugs, and a better therapeutic outcome can be achieved by overexpressing these miRNAs.

In the drug metabolism area, even less research has been so far pursued on miRNAs when compared with DNA methylation studies, and a review has shown that PK markers (including DMEs) are predicted to be regulated by a much smaller number of conserved miRNAs when compared with pharmacodynamic markers [56]. To our knowledge, the only available functional experiment includes the finding that CYP1B1 expression is inversely correlated with miR-27b that was found to be under-expressed in breast cancer tissue. MCF-7cells had high levels of miRNA-27b, and upon its inhibition, the activity of CYP1B1 increased using P450-Glo assay. Noteworthy, no correlation was found between levels of mRNA of *CYP1B1* and levels of miRNA-27b thus one can conclude that regulation takes place post-transcriptionally. CY1B1 is involved in the activation of procarcinogens and hydroxylation of 17b-estradiol that plays a role in the development and growth of estrogen dependent tissues [48].

Pharmacoepigenetics of drug metabolism & transport in breast cancer: *in silico* analysis & potential for further research

Candidate DMEs & transporters in breast cancer therapy

Most patients with breast cancer receive cytotoxic chemotherapy either in the adjuvant or neoadjuvant settings. One of the most frequently used regimens consists of four 2- or 3-week cycles of AC (doxorubicin plus cyclophosphamide) followed by four 2- or 3-week cycles of taxanes (paclitaxel or docetaxel) or six 3-week cycles of CAF (cyclophosphamide plus doxorubicin and 5-fluorouracil). Hormonal therapy with antiestrogenic agents is also a major component of therapy with tamoxifen being the most commonly used hormonal agent in estrogen receptor positive breast cancer [2,6]. Therefore, we chose to limit our analysis to these commonly used drugs. We searched the PharmGKB website for their PK pathways and extracted the genes of DMEs and transporters that had some pharmacogenetics evidence [57–61]. See Table 2 for the list of relevant genes that we evaluated.

CpG islands of genes of interest

As shown in Table 3, we searched for CpG islands in the genes of interest using the Epigenome database [62]. It turned out that many genes do not have CpG islands. Interestingly, *ABCB1* has two long CpG islands which may explain why it is the most commonly evaluated ABC transporter with an established role in acquired drug resistance. In addition, *CYP1B1* and *CYP2D6* are the only CYPs of interest with CpG islands, with that of *CYP1B1* being the longest by far. Note that one has to be aware of potential SNPs while designing PCR-based methylation analyses of regions of interest [63].

Based on previous reports indicating that DNA from tumor cells is released into the bloodstream [64]

Table 2. List of drugs and the relevant genes in breast cancer therapy that were included in the *in silico* analysis.

Drug	Genes with pharmacogenetic evidence
Doxorubicin	– *AKR1A1, AKR1C3* – *ABCB1, ABCC1, ABCC2, ABCG2* – *CBR1, CBR3, RALBP1, SLC22A16*
Cyclophosphamide	– *ALDH1A1* – *CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19* – *CYP3A4, CYP3A5*
5-fluorouracil	– *ABCC3, ABCC4, ABCC5, ABCG2* – *SLC22A7, SLC29A1* – *DPYD, DPYS*
Paclitaxel/docetaxel	– *ABCB1, ABCC1, ABCC2, ABCG2* – *CYP1B1, CYP2C8, CYP3A4, CYP3A5* – *SLCO1B3, SLC22A7*
Tamoxifen	– *CYP2D6* – *SULT1A1*

and that circulating DNA share genetic and epigenetic similarities with the DNA of original tumor cells [65], several studies investigated the role of serum DNA methylation in monitoring response to chemotherapy in breast cancer patients and showed promising data [66–70]. These studies, by showing that there is a tumor-specific expression of DMEs due to aberrant DNA methylation, provide a promising therapeutic strategy to cancer. Therefore, methylation analyses of genes of interest may be performed in both breast cancer tissues and peripheral blood as potential biomarkers of drug response.

Interestingly, all currently available literature on the pharmacoepigenetics of interindividual differences in drug response of cancer in general and breast cancer in particular evaluated the role of epigenetics in response to treatment such as survival and resistance (cell lines, cancer tissues and peripheral blood) [16,17,25–27,71,72]. To our knowledge, there is no toxicoepigenetic report on the association of epigenetic changes with cancer drug toxicity; we hence see promising research opportunities in this area. One could initially evaluate methylation of *ABC* transporters in peripheral white blood cells (WBCs) as potential predictive markers of myelotoxicity. This is because, and in contrast to germline genetic polymorphisms, epigenetic alterations in cancer tissues and/or peripheral circulating DNA that is shed from cancer tissue cannot be used to predict toxicity that is mainly related to the bone marrow and not to the tumor tissue itself. Results may explain the discrepancy between PK and pharmacogenetics candidate gene studies, and support the emerging role of epigenetic mechanisms. Potential reasons behind the lack of toxicoepigenetic studies on peripheral WBCs could be the scarcity of, or difficulty in designing studies that serially collect blood during therapy.

miRNA binding sites of genes of interest

We searched the target scan database [73] for sites in DMEs and transporters (conserved and poorly conserved) that can be potential targets for miRNAs. Note that the length of the 3′-UTR regions turned out to be directly correlated with the chance of the gene to be regulated by miRNAs. For instance and as shown in Table 4, there are many potential target sites for miRNA binding for all genes transcripts with the largest number being for *ABCG2* that has a 3′-UTR of 5223 bp (1834 at poorly conserved sites with 11 being at conserved sites). This is followed by *CYP2C19* (none at conserved sites), *CYP2B6* (one at conserved sites), *CYP1B1* (eight at conserved sites) and *CYP3A5* (six at conserved sites) among CYP enzymes.

Due to the large number of miRNAs that can potentially target the genes of interest, we then based our analysis on three reviews of miRNA expression in breast cancer and limited our search to those that were experimentally shown to be downregulated, upregulated or both in breast cancer tissues, cell lines or blood [21,22,25]. As shown in Table 4 with more details in Supplementary Table 1, we ended up with 54 candidate unique miRNAs or miRNA families of interest with 988 potential target sites. Interestingly, although currently available research on miRNA tar-

Table 3. Location and size of CpG islands of drug-metabolizing enzymes and transporters of interest in breast cancer therapy and list of variants within these islands when applicable.

Gene	Reference sequence	Gene start	Epigenome [62]	
			CpG islands start (length)	Variants within CpG islands
ABCB1	NC_000007.13	87133179	87257387 (1136) 87229419 (1048)	 rs9282564; rs2214102; rs3213619; rs2188524
ABCC1	NC_000016.9	16043434	16043027 (996)	rs504348
ABCC2	NC_000010.10	101542457	NA	NA
ABCC3	NC_000017.10	48712205	48712140 (660)	NA
ABCC4	NC_000013.10	95953705	95953195 (1355)	rs11568682; rs11568681; rs3751333
ABCC5	NC_000003.11	183637722	183734711 (1574)	NA
ABCG2	NC_000004.11	89011416	89079548 (1053)	NA
AKR1A1	NC_000001.10	46016455	46016226 (622)	NA
AKR1C3	NC_000010.10	5090958	NA	NA
ALDH1A1	NC_000009.11	75515578	NA	NA
CBR1	NC_000021.8	37442285	37442074 (795)	rs25678; rs1143663
CBR3	NC_000021.8	37506614	37507048 (1399)	NA
CYP1B1	NC_000002.11	38294746	38302590 (2103) 38300595 (1995)	rs162558; rs2567206; rs2551188 rs10012; rs2617266; rs1056827; rs57865060
CYP2A6	NC_000019.9	41349441	NA	NA
CYP2B6	NC_000019.9	41497138	NA	NA
CYP2C8	NC_000010.10	96796529	NA	NA
CYP2C9	NC_000010.10	96698350	NA	NA
CYP2C19	NC_000010.10	96522463	NA	NA
CYP2D6	NC_000022.10	42522501	42525356 (674) 42524592 (730)	rs267608311; rs28371706; rs267608308; rs28371704; rs267608309; rs267608310; rs267608289; rs267608306; rs1058164; rs267608303; rs267608304; rs267608305; rs5030865; rs267608302; rs267608301; rs1800716; rs3892097; rs267608290
CYP3A4	NC_000007.13	99354583	NA	NA
CYP3A5	NC_000007.13	99245813	NA	NA
DPYD	NC_000001.10	97543299	98386103 (741)	NA
DPYS	NC_000008.10	105391652	105478496 (988)	NA
RALBP1	NC_000018.9	9475530	9475530 (1329) 9478299 (784) 9535560 (602)	NA
SLC22A7	NC_000006.11	43265737	NA	NA
SLC22A16	NC_000006.11	110745890	110797547 (791)	NA
SLC29A1	NC_000006.11	44187242	44187045 (686) 44190708 (1287)	NA
SLCO1B3	NC_0000012.11	20963638	NA	NA
SULT1A1	NC_0000016.9	28616908	28634299 (1153) 28624861 (749)	NA

NA: Not applicable.

Table 4. Size of the 3′ untranslated regions of drug-metabolizing enzymes and transporters of interest in breast cancer therapy and number of conserved and poorly conserved sites for miRNAs binding.

Gene	Target scan human [73]				Target sites in DMEs and transporters of interest in breast cancer therapy	
	Ensembl transcript	3′-UTR length	Conserved sites	Poorly conserved sites	Conserved sites	Poorly conserved sites
ABCB1	ENST00000265724.3	1435	0	438	0	21
ABCC1	ENST00000399408.2	1793	19	771	0	40
ABCC2	ENST00000370449.4	2057	2	764	0	67
ABCC3	ENST00000427699.1	86	0	16	0	0
ABCC4	ENST00000412704.1	1746	3	652	0	45
ABCC5	ENST00000334444.6	1366	22	521	15	50
ABCG2	ENST00000515655.1	5223	11	1834	0	107
AKR1A1	ENST00000372070.3	95	0	34	0	0
AKR1C3	ENST00000380554.3	183	0	54	0	2
ALDH1A1	ENST00000297785.3	546	0	158	0	11
CBR1	ENST00000530908.1	1238	0	480	0	10
CBR3	ENST00000290354.5	54	0	18	0	6
CYP1B1	ENST00000260630.3	3586	9	1206	9	75
CYP2A6	ENST00000301141.5	258	0	90	0	3
CYP2B6	ENST00000324071.4	2962	1	1397	0	56
CYP2C8	ENST00000371270.3	2751	0	995	0	78
CYP2C9	ENST00000260682.6	363	0	114	0	7
CYP2C19	ENST00000371321.3	3282	0	1407	0	76
CYP2D6	ENST00000360608.5	75	0	31	0	3
CYP3A4	ENST00000354593.2	1173	0	468	0	24
CYP3A5	ENST00000339843.2	3098	6	1098	0	42
DPYD	ENST00000370192.3	1233	0	322	0	21
DPYS	ENST00000351513.2	434	1	161	0	6
RALBP1	ENST00000383432.3	2179	28	831	5	53
SLC22A7	ENST00000372589.3	813	9	403	6	31
SLC22A16	ENST00000439654.1	669	0	253	0	16
SLC29A1	ENST00000393844.1	2913	7	1341	0	64
SLCO1B3	ENST00000261196.2	3549	0	1140	0	66
SULT1A1	ENST00000350842.4	259	0	73	0	2

DME: Drug-metabolizing enzyme.

gets in breast cancer therapy has been based on analysis of potential candidates that bind to the 3′-UTR regions of genes of interest using various miRNA databases (*ABCB1* [38,39], *ABCC5* [40], *ABCC1* [41,42], *ABCG2* [43,44] and *CYP1B1* [48]), only three of the evaluated miRNAs (miRNA-128, miRNA-7, miRNA-27b) figured in the review articles, and there is hence plenty of room for further analyses.

As it was done with the cited research, one should evaluate the expression of the chosen target miRNAs in the tissue/cell line of interest. Ideally in breast cancer tissue studies, and because cytotoxic drugs are rarely given alone and the majority of patients receive a combination of chemotherapy such as cyclophosphamide with doxorubicin (adriamycin) and 5-fluorouracil [2,6], one should attempt to choose unique miRNAs that tar-

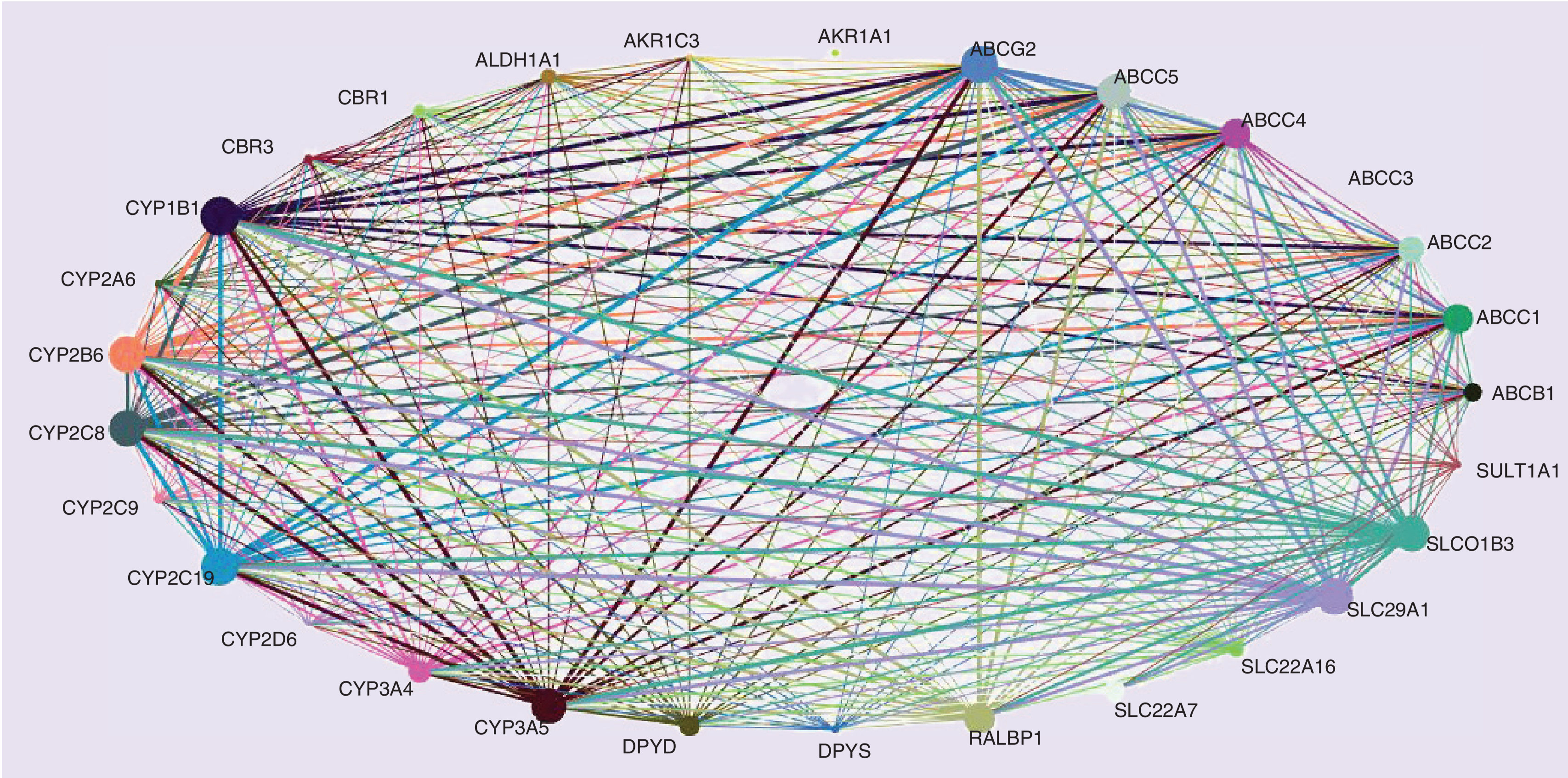

Figure 1. Network meta-analysis of shared miRNAs that can target 3′-UTRs of drug-metabolizing enzymes and transporters of interest in breast cancer therapy. Analysis was performed using MatLab software. Size of the circles correlates with the number of miRNA targets per gene. Thickness of the lines correlates with the number of shared miRNAs between two genes. Details are shown in Supplementary Table 1.

get genes involved in the disposition of one of the combination drugs only. As shown in Supplementary Table 1 and Figure 1 that depicts the extent of sharing of miRNA targets among these genes, it is almost impossible to do that except for two miRNAs: miR-191 and miR-128b as potential respective targets for *SLC29A1* and *ABCC4* that are unique to the 5-fluorouracil PK pathway [60]. These miRNAs may however not be dysregulated in the cells or population of interest, therefore one should build on available candidate or large microarray analyses of miRNA expression in tissues or populations of interest. For example, we have recently shown that in Lebanese breast cancer patients, there is a significant upregulation of miRNA-155, miRNA-21 and miRNA-148b and downregulation of miRNA-10b in cancer tissue when compared with normal adjacent tissue [74]. As shown in Supplementary Table 1, miRNA-155, miRNA-21 and miRNA-10b have several potential targets in DMEs and transporters of interest but with extensive overlap whereby, for example, *ABCC2*, *ABCG2* and *CYP2C19* are potential targets for all three, and the 3′-UTRs of many candidate genes of interest may be targets. Therefore, and in contrast to methylation studies whereby one can evaluate a region of interest of a candidate gene that is uniquely involved in the disposition of a certain cytotoxic drug, conclusions from pharmacoepigenetic miRNA evaluations in breast cancer patients cannot be narrowed down to one drug. We believe that, although this may seem like a limitation with clinical samples, such studies should be carried out, and recommendations should be tailored to the studied treatment regimens. We have recently evaluated the role of *CYP2B6* genetic polymorphisms in patients receiving cyclophosphamide combination therapy for breast cancer. We have shown that the studied SNPs were not significantly associated with myelotoxicity, yet *CYP2B6* haplotypes may predict treatment response whereby *CYP2B6*5/*6*, **6/*9* or **6/*6* haplotypes were associated with a significantly shorter time to disease recurrence [6]. In addition to *CYP2B6*, we could have evaluated more SNPs in additional candidate genes; nevertheless, a complementary strategy could also have also been attempted to associate the dysregulated miRNAs with treatment response.

Similarly to methylation analyses, several miRNA studies have been performed on circulating tumor cells and compared results to tissues proving that peripheral blood may be used as a source of epigenetic miRNA biomarkers [22,75]. For example, the serum expression of miRNA-155 that is of interest in the Lebanese population has been shown to predict response to therapy with taxane [76]. Conclusions from such an analysis would be relevant not only to taxanes therapy, but also to cyclophosphamide, doxorubicin (adriamycin) and 5-fluorouracil whose PK candidate genes can be targeted by miRNA-155. To our knowledge, and similarly to DNA methylation, there are currently no toxicoepigenetic miRNA studies in breast cancer.

Conclusion & future perspective

Pharmacoepigenetics entails alterations at the level of gene expression without any change in the gene itself. It includes DNA methylation, miRNA expression and histone modifications, and may affect the PK and pharmacodynamic properties of chemotherapeutic drugs. There is emerging evidence on the role of epigenetic biomarkers in breast cancer susceptibility and treatment [17].

In this manuscript, we have reviewed the available scant literature on DNA methylation and miRNA regulation of DMEs and drug transporters involved in the disposition of drugs commonly used in breast cancer and followed with an *in silico* analysis of the CpG islands and miRNA binding sites of genes of interest. We made the case that there is room for further research to include more genes and miRNAs despite the extensive sharing of miRNA targets by candidate genes of interest. We also stress on the role of peripheral blood as a source of pharmacoepigenetic biomarkers, and point out the lack of toxicoepigenetic studies in breast cancer.

Supplementary data

To view the supplementary data that accompany this paper, please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/pgs-2016-0081

Executive summary

- Epigenetic alterations include DNA methylation, miRNA expression and histone modifications, and may affect the pharmacokinetic and pharmacodynamic properties of chemotherapeutic drugs.
- There is emerging evidence on the role of epigenetic biomarkers in breast cancer susceptibility and treatment.
- There is little literature on DNA methylation and miRNA regulation of drug-metabolizing enzymes and drug transporters involved in the disposition of drugs commonly used in breast cancer.
- *In silico* analysis shows that there is room for further research to include more genes and miRNAs as well as toxicoepigenetic studies in breast cancer.
- Peripheral blood is a clinically important source for pharmacoepigenetic biomarkers.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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