



## Review Article

# DNA Methylation and Anticancer Drug Resistance in Gynecological Tumors



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### Abstract

DNA methylation is essential for regulating tissue-specific gene expression, genomic imprinting, X chromosome inactivation and retroviral element silencing. The transformation from normal to cancer cells is accompanied by changes in DNA methylation resulting in the activation of oncogenes and inactivation of tumor suppressor genes. This process is regulated by methylation and contributes to the support and development of tumors. Epigenetic modifications account for the development of resistance in cancer cells treated with anticancer drugs. Dysregulated signaling pathways involved in tumor drug resistance include the Wnt canonical and non-canonical pathways and the PI3K/PTEN/AKT/mTOR pathway. This review considers the mechanisms and specific methylated biomarkers that participate in such resistances and how resistance to individual treatments for breast, ovarian, uterine and cervix tumors are introduced.

**Keywords:** Epigenetics; Breast cancer; Ovarian cancer; Endometrial cancer; Cervical cancer; Wnt; PI3K/PTEN/AKT/mTOR; DNA damage repair; DNA methyltransferase; TET.

**Abbreviations:** ASS1, argininosuccinate synthetase 1; BAX, B-cell lymphoma-2 associated X; BC, breast cancer; BCL2, B-cell lymphoma; BRCA1, breast cancer 1; CCN, cyclin; CDKN, cyclin dependent kinase; DME, drug metabolizing enzymes; DNMT, DNA methyltransferase; EGFR, epidermal growth factor receptor; EOC, epithelial ovarian cancer; ER $\alpha$ , estrogen receptor  $\alpha$ ; FAS, cell surface death receptor gene; GST, glutathione S transferase; HDAC, histone deacetylase; HER2, human epidermal growth factor receptor 2; HERV, human endogenous retrovirus; HGSOC, high-grade serous ovarian cancer; HOX, homobox; hSulf-1, human sulfatase-1; ID4, DNA-binding inhibitor 4; IL, interleukin; MAL, myelin and lymphocyte; MDR1, multidrug resistance 1; MGMT, O<sup>6</sup>-methylguanine-DNA methyltransferase; MGP, matrix gla protein; miR, microRNA; MMR, DNA mismatch repair; MRP, multidrug resistance proteins; MSH2, mismatch protein; NAGA, alpha-N-acetylgalactosaminidase; NAT, N-acetyltransferase; OC, ovarian cancer; OCCA, ovarian clear cell carcinoma; OCT, octamer; OXCT1, 3-Oxoacid CoA transferase 1; PARP, poly (ADP-ribose) polymerase; PDX, patient-derived xenograft; PGK1, phosphoglycerate kinase 1; P-GP, P-glycoprotein; PI3K, phosphatidylinositol 3-kinase; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PKB, kinase B; PLK, polo-like kinases; PSAT1, phosphoserine aminotransferase 1; PTEN, phosphatase tensin homolog; RassF1A, Ras association domain family 1A; RTK, receptor tyrosine kinase; SALL2, spalt-like transcription factor 2; SEPT9, Septin 9; SIRT1, sirtuin 1; SLC, solute carrier; SLFN11, Schlafen-11; SOCS, suppressor of cytokine signaling; SRC, steroid receptor coactivator; STAT3, signal transducer and activator of transcription 3; TET, ten-eleven translocation; TGF $\beta$ 1, transforming growth factor B1; TLR, Toll-like receptor; TMEM88, transmembrane protein 88; TNBC, triple-negative breast cancer; TNF, tumor necrosis factor; TOR, target of rapamycin; TRAF6, TNF-associated factor 6; TRAIL, TNF-related apoptosis-inducing ligand; TRIB2, tribbles 2; UCHL1, ubiquitin C-terminal hydrolase L1; Upa, urokinase; URFH1, ubiquitin-like, containing PHD and RING finger domains 1; ZNF582, zing finger 582.

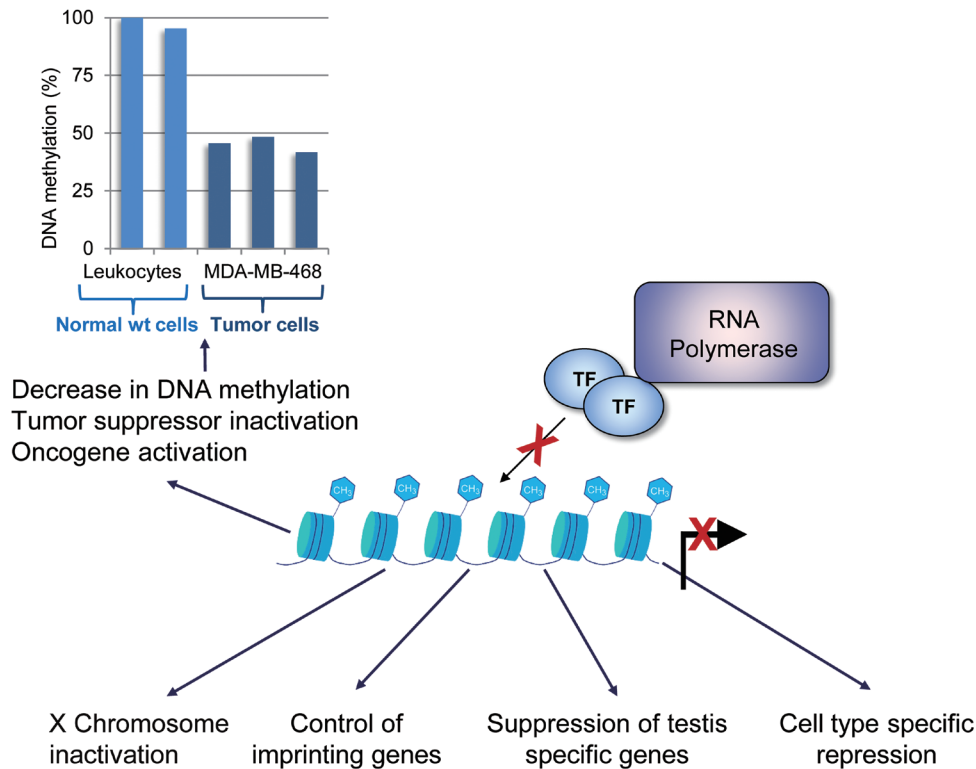
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### Introduction

Based on Baldwin’s suggestion at the end of the nineteenth century of the “correct” allele choosing a new environment, which leads to a permanently changed evolutionary development within that environment,<sup>1,2</sup> Waddington suggested the term epigenetics. He described a context in which a characteristic acquired within a total population in response to an environmental stimulus might be inherited in the absence of DNA mutations.<sup>3,4</sup> This process involved a phenotypic modification occurring through the alteration of gene expression; however, with no modification in the actual gene DNA sequence. Despite initial opposition to the theory, epigenetics has become a central aspect of genetic studies. It plays a role in numerous processes, for example, cell type-specific gene inactivation (Fig. 1). It is important in the initiation and development of cancers and the development of anticancer drug resistance. The epigenetic modification of importance is DNA methylation and its involvement in nucleosome repositioning, histone post-translational modification and post-transcriptional gene regulation by microRNAs (miRNAs).<sup>5</sup>

DNA methylation, which was first identified in 1944,<sup>6</sup> involves the DNA cytosine residue rather than the adenine residue that is rarely methylated in humans. Cytosine methylation is catalyzed by the DNA methyltransferase (DNMT) family of enzymes that transfer a methyl group from S-adenosyl-methionine to the fifth carbon of a cytosine residue to form 5-methylcytosine (5mC). These enzymes include: (1) DNMT1 which functions in DNA replication by binding to the newly synthesized, unmethylated DNA daughter strand to ensure that it is similarly methylated to the par-



**Fig. 1. DNA methylation and its role in benign and malignant cells.** ELISA was performed to assess the percentage of DNA methylation in leukocytes and MDA-MB-468 cells. Then, the measurements were depicted in a bar chart. The results show DNA methylation in the metastatic adenocarcinoma breast cell line MDA-MB-468 with a 50 % lower DNA methylation than in normal leukocytes. These results highlight a decrease in DNA methylation in cancer cells. The binding of RNA polymerase and transcription factor (TF) to methylated DNA is inhibited by methyl groups (depicted on a DNA strand wrapped around histones), leading to the inhibition of RNA transcription. DNA methylation plays an important role in processes, such as the inactivation of the X chromosome, imprinting genes, testis-specific genes, and cell type-specific genes.

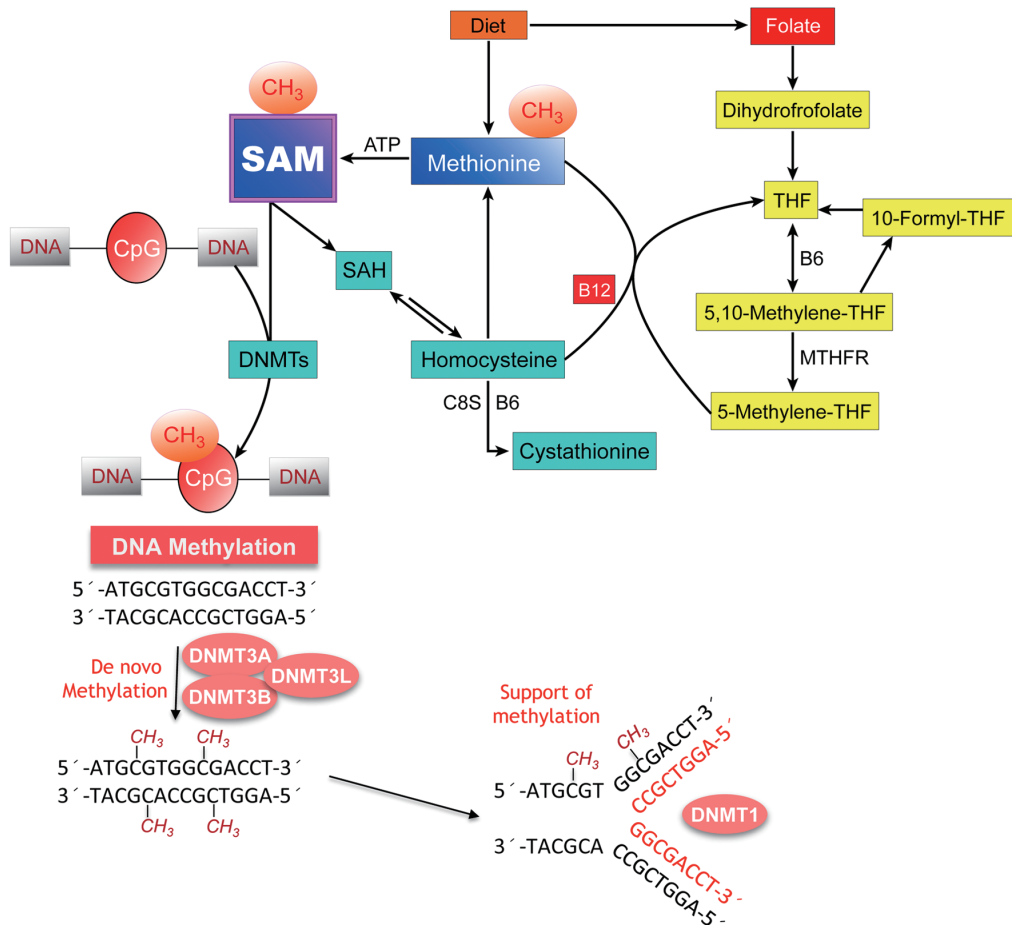
ent strand.<sup>7</sup> In addition, DNMT1 can act as a maintenance enzyme due to its ability to repair DNA methylation.<sup>8</sup> The recruitment of DNMT1 to cytosine depends upon the binding of URFH1 (ubiquitin-like, containing PHD and RING finger domains 1). Failure to do so means no methylation. In addition, DNA methylation is further regulated by the arginine methyltransferase PRMT6 through its ability to methylate the arginine residue at position 2 of histone 3 (H3R2me2a) in the nucleosome complex. The presence of H3R2me2a blocks the binding of URFH1 and hence cytosine methylation;<sup>9</sup> (2) DNMT2 (TRdnmt), which is a DNMT homolog that does not methylate DNA; and (3) DNMT3a and DNMT3b that methylate DNA with approximately 75% of CpG dinucleotides being methylated in somatic cells.<sup>10</sup> These enzymes can cooperate with histone-modifying enzymes that act by either adding or removing either of both histone markers to result in repression of the gene region.<sup>11</sup> However, DNMT3a is expressed in most differentiated tissues and DNMT3b is poorly expressed,<sup>12</sup> and knockout studies on mouse embryos have indicated that DNMT3b is primarily important in embryo development.<sup>13</sup> An additional DNMT3 (DNMT3L) does not have a catalytic function but seems to associate with DNMT3a and DNMT3b stimulating their methyltransferase activity. In addition, DNMT3L is needed for maternal and paternal genomic imprinting, X chromosome compaction and retrotransposon methylation (Fig. 2).<sup>11</sup>

DNA methylation occurs on cytosines present at the CpG sites of the DNA that are spread throughout the genome. It does occur at those cytosines present in the CpG islands, for instance, stretches

of DNA of demethylation 300–3,000 base pairs long have a higher CpG density than the rest of the genome.<sup>14–16</sup> Expanses of CpG islands in non-methylated stretches have been termed large valleys or canyons and appear to be present throughout the mammalian genome.<sup>17,18</sup> Overall, 70% of promoters present adjacent to transcription start sites of genes appear to contain a CpG island.<sup>19,20</sup> Therefore, stable silencing of genes can be achieved by the methylation of the CpG islands associated with the promotor regions.<sup>21</sup>

In general, DNA methylation is essential for regulating tissue-specific gene expression, genomic imprinting, X chromosome inactivation and, importantly, retroviral element silencing (Fig. 1). Overall, 70% of gene promoters are contained within CpG islands including those of housekeeping genes.<sup>22</sup>

Although DNA methylation appears to be stabilized in postmitotic cells once an embryo has fully developed, cancer cell initiation will reactivate DNA methylation or demethylation in these cells. DNA activity is modified by methylation and by demethylation, which is a less well-understood process. This activity is initiated by the ten-eleven translocation (TET) enzyme family that includes TET1, TET2 and TET3.<sup>23</sup> They are  $\alpha$ -ketoglutarate-dependent dioxygenases involved in the TET-mediated oxidation of 5mC and 5-hydroxymethylcytosine (5hmC), the alpha-ketoglutarate being converted into succinate and CO<sub>2</sub>. The products of this activity, 5mC and 5hmC, are then converted into 5-formylcytosine (5fc) and 5-carboxycytosine (5caC).<sup>24,25</sup> The produced 5hmC is a stable epigenetic modification and accounts for 1–10% of the 5mC.<sup>24</sup> 5mC and 5hmC are then oxidized into other cytosine

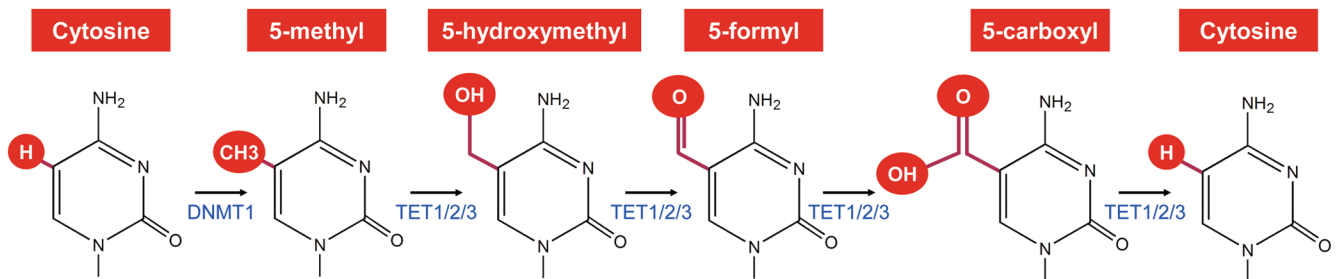


**Fig. 2. Production of methyl groups and DNA methylation.** Synthesis of the amino acid methionine produces SAM, the main donor for DNA methylation. Two cofactors are necessary: vitamin B12 and folic acid. Varying amounts of these cofactors in food lead to higher or lower cellular DNA methylation. The intake of folic acid and vitamin B12 promotes erythrocyte formation. DNMT3A, DNMT3B, and DNMT3L are responsible for the establishment of the first DNA methylation pattern. This *de novo* DNA methylation, passed on by the parent to the progeny, establishes key epigenetic modifications that are essential for cellular differentiation and embryonic development. DNMT1 supports DNA methylation by copying the pattern from the old DNA strand by transferring methyl groups to the newly synthesized strand. DNMT, DNA methyltransferase; SAH, S-adenosyl-homocysteine; SAM, S-adenosyl-methionine; THF, tetrahydrofolate.

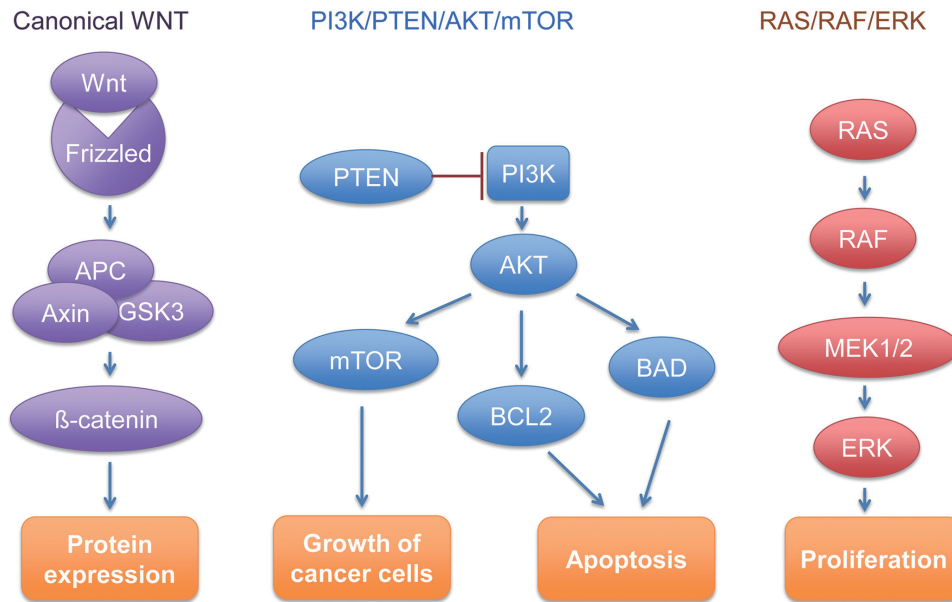
forms, for example, 5fc and 5caC,<sup>26</sup> which are then identified and excised by thymine DNA glycosylase, repaired through the base-excision repair system and subsequently replaced by cytosine (Fig. 3).<sup>27</sup> The role of DNMT and TET proteins compose the control of the methylation of the CpG islands associated with the promoter regions;<sup>21</sup> therefore, permitting the stable flow of epigenetic in-

formation between cell generations including gene expression in embryonic and differentiated tissues.

The homeodomain-containing protein NANOG is essential to establish the ground state of pluripotency during somatic cell reprogramming. This protein has a physical association with TET1 and TET2, which leads to an enhanced reprogramming efficien-



**Fig. 3. DNA demethylation pathway.** Demethylation is performed at the 5' positions on the pyrimidine ring of cytosine 5' to guanine within the DNA (Fig. 2). The TET enzymes catalyze the hydroxylation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), then the oxidation of 5hmC to 5-formylcytosine (5fC), 5fC to 5-carboxycytosine (5caC), and finally 5caC to cytosine. For clarity, only the single modified cytosines are depicted. TET, ten-eleven translocation.



**Fig. 4. Three signaling pathways.** The activation of the canonical Wnt, PI3K/PTEN/AKT/mTOR, and RAS/RAF/ERK signaling pathways with the essential components. APC, adenomatous polyposis coli; BAD BCL2, associated agonist of cell death; Bcl-2 agonist of cell death; BCL2, anti-apoptotic B cell lymphoma 2; ERK, extracellular-signal regulated kinase; GSK-3, glycogen synthase kinase-3; MEK, mitogen-activated protein kinase; PI3K, phosphatidylinositol-3-kinase; PTEN, phosphatase and tensin homolog; RAF, rapidly accelerated fibrosarcoma; RAS, rat sarcoma; TOR, target of rapamycin.

cy.<sup>28</sup> In addition, Costa *et al.* determined 27 protein interaction partners of NANOG. Furthermore, they indicated that TET1 was recruited by NANOG and enhanced key reprogramming target gene expression. NANOG is thought to function together with additional proteins, for example, PO5F1 and SOX2 in embryonic stem cells, which is an important factor in tumor cells where it is highly expressed.<sup>29</sup> NANOG appears to function as an oncogene leading to carcinogenesis since its high expression can be used as a marker of poor prognosis.<sup>29-31</sup> In addition, the expression of the NANOG p8 protein is important in cancer stem cells.<sup>32</sup>

Recently, an uncharacterized protein (QSER1) was suggested as a TET1 cobinding protein.<sup>33</sup> When competing for DNA binding sites in competition with DNMT3A and DNMT3B, they are mutually dependent.

### Major signaling pathways involved in tumor development and growth

Major signaling pathways involved in tumor drug resistance include the Wnt canonical and non-canonical pathways and the PI3K/PTEN/AKT/mTOR pathway. These can be regulated by methylation to contribute to the support and development of tumors.

#### *Wnt canonical and non-canonical signaling pathways*

The Wnt family contains a variety of secreted cysteine-rich lipoproteins that activate several signaling pathways through their binding to frizzled receptors and coreceptors on the cell membrane.<sup>34-37</sup> These derived signals participate in key cellular functions that include proliferation, differentiation, migration, genetic stability and apoptosis. Two Wnt pathways are involved: the canonical pathway that relies on the involvement of B-catenin (Fig. 4) and the non-canonical pathway that does not rely on it. The latter is activated

by the Wnt/planar cell polarity and Wnt/Ca<sup>2+</sup> pathways.<sup>37-41</sup> Van Amerongen *et al.*<sup>42</sup> proposed the possibility of an integrated Wnt pathway in which there was a combination of the canonical and non-canonical pathways that lead to multiple inputs at the Wnt receptor binding and downstream intracellular responses. Consequently, a variety of tumors that include breast and ovarian show a deregulated methylation pattern in the Wnt pathway.<sup>43</sup>

#### *PI3K/PTEN/AKT/mTOR signaling pathway*

Phosphatidylinositol 3-kinase (PI3K) or AKT, a serine/threonine protein kinase that is known as protein kinase B, and the target of rapamycin (mTOR) are major components in this pathway (Fig. 4). They are activated by upstream tyrosine kinases together with, for example, hormones and mitogenic factors. The signaling pathway is important in a range of cellular processes including general cell metabolism, cell proliferation, protein synthesis for cell growth, cell motility and apoptosis.<sup>44</sup> PI3K is composed of three classes of which class 1 is important in cancer.<sup>45</sup> Class 1 PI3K is activated by either receptor tyrosine kinases or G protein-coupled receptors. They are primarily linked to the conversion of phosphatidylinositol 4,5-bisphosphate (PI4, 5P2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3). The central pathway point (AKT) is activated by PIP3 resulting in its binding to the cell membrane and acting downstream in cellular processes that are linked to cell survival, growth and proliferation.<sup>46,47</sup> mTOR is an important protein that can act upstream and downstream of AKT.<sup>48</sup> mTOR is active in the targeting of rapamycin complexes (*e.g.*, TORC1 and TORC2) and regulates a number of cellular processes including the synthesis of proteins for cell growth and proliferation.<sup>48</sup> DNA methylation, and therefore, modification of this pathway and an imbalance in oncogenes, lead to cancer cell maintenance and development and drug resistance. The PI3K/PTEN/Akt/mTOR signaling pathway is deregulated in numerous cancers leading to altered cellular processes, which makes this

axis an attractive target for therapeutic manipulations. Upregulated DNMT induces hypermethylation of components of this oncogenic pathway, for example, the inactivation of the negative regulator and tumor suppressor gene phosphatase tensin homolog (PTEN). Reduced PTEN expression is associated with activation of AKT leading to the aberrant deregulation of the pathway to confer tumor growth and drug resistance.<sup>49,50</sup>

#### **Other signaling pathways**

To date, numerous other signaling pathways involved in tumor drug resistance that are deregulated by DNA methylation have been described. Among others, the MAPK pathway leads to cell proliferation, differentiation, migration, senescence and apoptosis<sup>51</sup> whilst DNA damage repair pathways support genomic integrity and DNA replication.<sup>52</sup> Cell adhesion/tight junction pathways link key signaling pathways in cell proliferation, transformation and metastasis<sup>53</sup> and the NOTCH pathway influences differentiation, proliferation and apoptotic cell fates.<sup>54</sup>

#### **Major additional signaling pathways involved in DNA methylation**

Important signaling pathways involved in DNA methylation have been described in a review by Hegde and Joshi.<sup>55</sup> A brief description of these pathways follows.

##### **Ras/AP-1 signaling pathway**

The RAS superfamily of GTPases (Fig. 4) regulates cell proliferation, apoptosis and cell migration. Increased expression of RAS plays an important role in the epigenetic silencing of several genes in human tumors. Since the DNMT1 promoter contains several AP1 sites, the RAS signaling pathway regulates DNMT1 via AP1. Aberrant expression of RAS in breast cancer (BC) results in increased DNA methylation as has been well documented.<sup>55</sup>

##### **JAK1/STAT3 signaling pathway**

Signal transducer and activator of transcription 3 (STAT3) is a transcription factor (TF) that, on phosphorylation by JAK1 tyrosine kinase, forms homo or heterodimers to modulate cell proliferation, apoptosis and cell motility. The binding of STAT3 to the DNMT1 promoter in BC cells indicates its crucial role in epigenetic changes during tumorigenesis and metastasis.<sup>55</sup>

#### **Other signaling pathways**

To date, numerous other signaling pathways involved in DNA methylation have been reported. These include retinoblastoma and TP53 signaling that regulate DNMT1-mediated gene promoter methylation.<sup>55</sup>

#### **Abnormal methylation of apoptosis-related genes in cancer drug resistance**

Apoptosis plays a key role in the control of cancer cell growth.

It can be triggered either by extrinsic receptor stimulation or intrinsic mitochondria-mediated signaling. The extrinsic pathway involves, for example, cell surface death receptor gene (FAS), tumor necrosis factor (TNF) or TNF-related apoptosis-inducing ligand (TRAIL), which activate caspase-8. Then, activated caspase-8 either directly cleaves or activates caspase-7 and caspase-3, promoting apoptosis. However, the intrinsic pathway leads to the activation of B cell lymphoma-2 (BCL2) associated X (BAX) at the mitochondrial outer membrane leading to the release of different apoptosis-mediating molecules, such as cytochrome c, which activates caspase-9. Then, caspase-9 cleaves and activates caspase-3 and caspase-7 to promote apoptosis. In addition, the tumor suppressor p53, which is a key regulator of apoptosis, has an essential role in apoptosis. At the transcriptional level, p53 either upregulates (*e.g.*, BAX) or reduces the expression of BCL-2, which antagonizes BAX. A high ratio of BCL-2 to BAX protein confers a poor prognosis with decreased rates of complete remission and overall survival.<sup>56</sup> Therefore, DNA methylation, which mediates the downregulation of genes involved in apoptosis, is an essential mechanism through which tumor cells avoid apoptosis and survive. As described in the following sections or the detailed review by Hervouet *et al.*,<sup>57</sup> numerous genes implicated in apoptosis may be aberrantly methylated in cancer. This is frequently associated with chemoresistance.

#### **DNA methylation and drug resistance in cancer cells**

As mentioned previously, CpG islands are associated with gene promoter regions<sup>21</sup> that are stabilized by methylation in post-mitotic cells. In such healthy cells, the CpG islands tend to be hypomethylated and the remaining part of the genome tends to be methylated. In cancer cells; however, a reverse process is observed where the CpG islands are hypermethylated. The result of this process is the blocking of key genes by CpG island hypermethylation of promoter regions in cancer cells leading to diminished gene expression relevant to normal cell performance. Cancer types have specific groups of these hypermethylated CpG islands, which are known as CpG island methylator prototype (CIMP) that are specific for a given tumor and are different between cancer types. One of the first CIMP examples was identified in colorectal cancer.<sup>56</sup>

This could lead to tumor cell resistance to trastuzumab, anti-estrogen, doxorubicin and tamoxifen in BC and radiation in cervical cancer. In addition, ovarian cancer (OC) cells show resistance to cisplatin, carboplatin, gefitinib and paclitaxel.<sup>49</sup> Romero-Garcia *et al.* reviewed the effects of hypomethylation of promoter genes leading to increased gene expression. In this case, resistance is associated with tamoxifen, doxorubicin, paclitaxel, cyclophosphamide, docetaxel, doxorubicin and radiation for BC and carboplatin and cisplatin for OC (Table 1).<sup>49,58-81</sup> The course of action of doxorubicin in the different pathways leading to cell death and cell growth arrest is shown in Figure 5.<sup>82</sup> Several tumors, such as lung, breast, prostate, colon, gastric, and OCs, among others, exhibit a pattern of deregulated methylation in cancer-associated pathways.<sup>35,58,83</sup>

#### **Drug transport**

Anthracyclines, such as doxorubicin, and taxanes, such as paclitaxel or carboplatin, are highly effective drugs that are used in the treatment of BC and other cancers, drug transports limit their

**Table 1. Hypermethylated and hypomethylated promoters or genes and drug resistance (after<sup>49</sup>)**

Authors	Hypermethylated		
	Tissue	Promoter or gene	Drugs
Palomeras <i>et al.</i> <sup>61</sup>	Breast	TGFB1	Trastuzumab
Zhang <i>et al.</i> <sup>58</sup>	Breast	ER $\alpha$	Anti-estrogen
Ponnusamy <i>et al.</i> <sup>62</sup>	Breast	MSH2	Doxorubicin
Tuo <i>et al.</i> <sup>63</sup>	Breast	MGP	Doxorubicin
De Marchi <i>et al.</i> <sup>64</sup>	Breast	PSAT1	Tamoxifen
Kim <i>et al.</i> <sup>65</sup>	Cervix	SOCS 1, SOCS 3	Radiation
Wu <i>et al.</i> <sup>66</sup>	Cervix	ZNF582	Radiation
Jin <i>et al.</i> <sup>67</sup>	Ovarian	UCHL1	Cisplatin
Yang <i>et al.</i> <sup>59</sup>	Ovarian	OXCT1	Cisplatin
Prieske <i>et al.</i> <sup>68</sup>	Ovarian	BRCA 1	Cisplatin
Deng <i>et al.</i> <sup>69</sup>	Ovarian	miR-199a-3p	Cisplatin
Tian <i>et al.</i> <sup>60</sup>	Ovarian	hMSH2	Cisplatin
Gao <i>et al.</i> <sup>70</sup>	Ovarian	RassF1A	Cisplatin/Placitaxel
Ha <i>et al.</i> <sup>71</sup>	Ovarian	NAGA	Cisplatin
Kritsch <i>et al.</i> <sup>72</sup>	Ovarian	TRIB2	Cisplatin
Zhang <i>et al.</i> <sup>73</sup>	Breast	ID4	Tamoxifen
Chen <i>et al.</i> <sup>74</sup>	Breast	ERp29/MGMT	Radiation
Hu <i>et al.</i> <sup>75</sup>	Breast	miR-663	Docetaxel
Chekhun <i>et al.</i> <sup>76</sup>	Breast	MDR1, GSTpi, MGMT, Upa	Doxorubicin
Pan <i>et al.</i> <sup>77</sup>	Ovarian	SERPINE1	Carboplatin
De Leon <i>et al.</i> <sup>78</sup>	Ovarian	TMEM88	Carboplatin
Li <i>et al.</i> <sup>79</sup>	Ovarian	BCRA1, SIRT1, EGFR	Cisplatin
Iramaneerat <i>et al.</i> <sup>80</sup>	Ovarian	HERV	Cisplatin
Lee <i>et al.</i> <sup>81</sup>	Ovarian	MAL	Cisplatin

BRCA1, BC; ER $\alpha$ , estrogen receptor  $\alpha$ ; EGFR, epidermal growth factor receptor; GSTpi, glutathione S transferase pi; HERV, human endogenous retrovirus; ID4, DNA binding inhibitor 4; MAL, myelin and lymphocyte; MDR1, multidrug resistance 1; MGMT, O6-methylguanine-DNA methyltransferase; MGP, matrix gla protein; MSH2, mismatch protein; miR, microRNA; NAGA, alpha-N-acetylgalactosaminidase; OXCT1, 3-Oxoacid CoA transferase 1; PSAT1, phosphoserine aminotransferase 1; RassF1A, Ras association domain family 1A; SIRT1, sirtuin1; SOCS, suppressor of cytokine signaling; TGFB1, transforming growth factor B1; TMEM88, transmembrane protein 88; TRIB2, tribbles 2; UCHL1, ubiquitin C-terminal hydrolase L1; Upa, urokinase; ZNF582, zing finger 582.

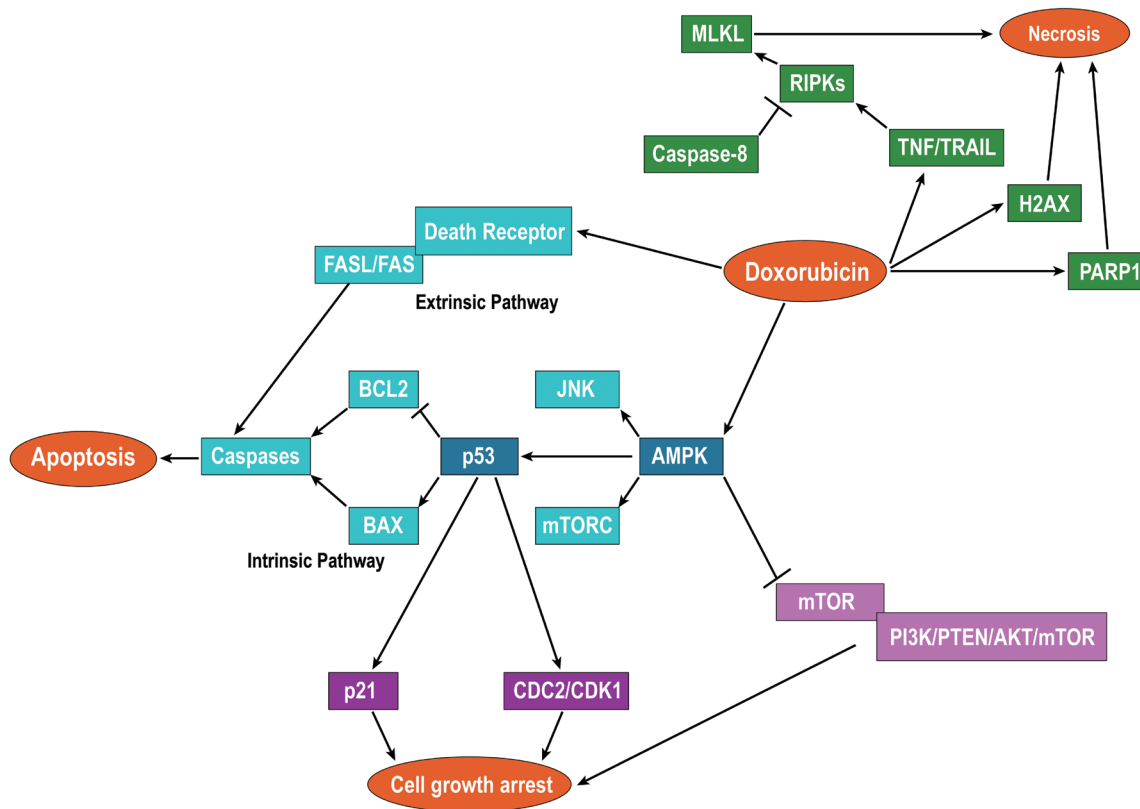
clinical efficacy. On entering the body, anticancer drugs will pass through a series of complex processes that include drug transport and metabolism. Tumors can either be intrinsically resistant to these agents or acquire resistance upon exposure to chemotherapeutic drugs. Drug resistance, whether intrinsic or acquired, is assumed to cause therapy failure in >90% of patients with metastatic tumors.<sup>84</sup>

Drug transporters are ubiquitous membrane-bound proteins regulating the movement of drugs and endogenous metabolites into and out of the cell. In mammals, they are expressed primarily in the liver, intestines, blood-brain barrier, blood-testis barrier, placenta and kidneys,<sup>85</sup> maintaining homeostasis and mediating processes that are important for pharmacokinetics. They are divided into the ATP binding cassette (ABC) family including P-glycoprotein, BC resistance protein, multidrug resistance proteins (MRPs) and the solute carrier (SLC) family including organic anion and cation transporters.<sup>86</sup> ABC drug transporters are closely connected to metabolic pathways and using ATP, actively pump endogenous

metabolites and cytotoxic drugs out of tumor cells and SLC transporters mediate the influx of cytotoxic drugs into cells.<sup>87</sup> Therefore, they control the influx and efflux of chemotherapeutic drugs, modulating the intracellular drug concentration and therefore, determining the therapeutic efficacy and the success or failure of patient treatment.

Of the ABC transporters, MRP1, 2 and 4 are involved with platin transport, MRP1 transports only oxaliplatin and MRP2 and 4 transport cisplatin and oxaliplatin. Furthermore, doxorubicin and irinotecan affect the expression of MRPs in a promoter methylation-dependent manner.<sup>88</sup> In addition, the ATPases (*e.g.*, ATP7A and ATP7B) transport cisplatin, oxiplatin and carboplatin.<sup>89</sup>

The overexpression of ABC drug transporter may be caused by epigenetic changes that are essential for the acquisition of drug resistance and are associated with resistance to numerous chemotherapeutic agents. Early work on DNA methylation levels and drug resistance dates from the mid-1980s. For example, Nyce<sup>90</sup> reported the effects of drug-induced methylation in lung adenocar-



**Fig. 5. Role of doxorubicin in cellular pathways.** Doxorubicin initiates the extrinsic pathway of apoptosis by FASL/FAS, activating caspase-8, -3, -6 and -7 and the intrinsic pathway by upregulating AMPK so leading to the upregulation of p53, JNK and mTORC1. The inhibition of anti-apoptotic BCL2 and the increase in pro-apoptotic BAX lead to the activation of caspases-3, -6 and -7. Upregulated p21 inhibits the CDC2/CDK1 ratio leading to cell growth arrest. The necrosis pathway is initiated by the activation of either TNF or TRAIL and the inhibition of caspase-8. RIPK1 activates RIPK3, leading to the activation of MLKL. Upregulated PARP-1 and H2AX decrease glycolysis to induce necrosis. Upregulated AMPK inhibits mTOR in the PI3K/PTEN/AKT/mTOR leading to the inhibition of cancer growth. These pathways are abbreviated shown because of the clarity, but a detailed description is given by Meredith *et al.*<sup>82</sup> Other chemotherapeutic agents, such as carboplatin, act in a similar way. DNA methylation of the upregulated components of the pathway leads to inhibition of cell death and tumor growth so inducing chemoresistance. AMPK, activated protein kinase; BAX, BCL-2-associated X; BCL-2, B cell leukemia/lymphoma 2; CDC2, cell-division cycle 2; CDK, cyclin-dependent kinase; FASL, FAS ligand need to explain; JNK, c-Jun N-terminal kinase; MLKL, mixed-lineage kinase domain-like protein; mTORC1, mammalian target for rapamycin complex 1; PARP-1, poly (ADP-ribose) polymerase-1; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; RIPK, receptor-interacting serine/threonine protein kinase; TNF- $\alpha$ , tumor necrosis factor alpha; TRAIL, TNF-related apoptosis-inducing ligand.

cinoma and rhabdomyosarcoma cells. Pulse exposure to a range of antitumor agents affecting different aspects of the tumor cells included etoposide, nalidixic acid, doxorubicin, vincristine, vinblastine, colchicine, cisplatin, hydroxyurea, 1-beta-D-arabinofuranosylcytosine, 5-fluorouracil, 5-fluorodeoxyuridine and methotrexate, which all led to drug-induced DNA hypermethylation. That this was not a cell culture-specific event was confirmed by its occurrence in leukemic patients undergoing treatment with high-dose 1-beta-D-arabinofuranosylcytosine and hydroxyurea. Subsequent studies have shown that similar results occur in cancer drug resistance.

Epigenetic regulation of organic cation transporters has been shown for OCT1 (octamer, SLC22A1),<sup>91</sup> OCT2 (SLC22A2),<sup>92</sup> OCT3 (SLC22A3),<sup>93</sup> MATE1 (SLC47A1),<sup>94</sup> OCTN1 (SLC22A4)<sup>95</sup> and OCTN2 (SLC22A5).<sup>96</sup> An example of anticancer drug transport can be observed through studies of platin drugs. Therefore, cisplatin is a substrate for OCT1 and OCT2 and oxaliplatin is a substrate for OCT2 and OCT3.<sup>89</sup> Variations in the methylation of these transporters can lead to a drug resistance through the modified availability of the anticancer drug employed. In an early

study, Schaeffeler *et al.*,<sup>91</sup> observed a significant downregulation of OCT1 protein expression in hepatocellular carcinoma compared with normal adjacent tissue due to increased *OCT1* methylation. Qu *et al.*<sup>96</sup> employed methylation-specific PCR and bisulfite genomic sequencing to demonstrate that the degree of individual methylated CpG sites within *OCTN2* was inversely correlated with its levels of activity in different cancer cells; therefore, resulting in the reduced uptake of oxaliplatin. Furthermore, this reduced activity could be reversed by the application of dichloroacetate, which increased *OCTN2* expression and enhanced oxaliplatin uptake. Subsequently, Buelow *et al.*<sup>95</sup> determined that an increased basal *OCTN1* methylation was linked with a decreased cytarabine uptake in acute myeloid leukemia cell lines. Pre-treatment with hypomethylating agents, such as 5-azacytidine and decitabine led to increased cellular uptake of cytarabine with an associated increase in cellular sensitivity to cytarabine.

To circumvent the action of drug transporters, alternative strategies have been reported, for example, the application of monoclonal antibodies directed against P-glycoprotein and liposome-encapsulated drugs.<sup>97</sup>

## Metabolism

Drug metabolism includes a modification of anticancer drugs through catalysis by drug-metabolizing enzymes (DMEs), such as phase I and II DMEs. The expression of DMEs is epigenetically regulated, for instance, by DNA methylation. Habano *et al.*<sup>98</sup> reported that some DME genes were regulated by DNA methylation, which permitted inter- and intra-individual differences in drug metabolism. An analysis of the DNA methylation landscape facilitated clarification of the role of DNA methylation in the regulation of DME genes leading to potential tumor suppression.

Cytosine DNA methylation of DME genes can lead to their activation, metabolic inactivation and, finally, chemotherapy resistance.<sup>99</sup> There are two groups of DMEs, such as phase I (functionalization) and II (conjugation) reactions. Phase I reactions concern the redox or hydrolysis of the drug to either activate or detoxify it. This involves cytochrome P450 enzymes (P450s), flavin-containing monooxygenases, alcohol dehydrogenases and aldehyde dehydrogenases.<sup>100</sup> Phase II are transferases, such as UDP-glucuronosyltransferases, sulfotransferases, glutathione S-transferases (GSTP1) and N-acetyltransferases (NAT1).<sup>101</sup> Therefore, the various breakdown components are converted into water-soluble products that can be readily excreted.

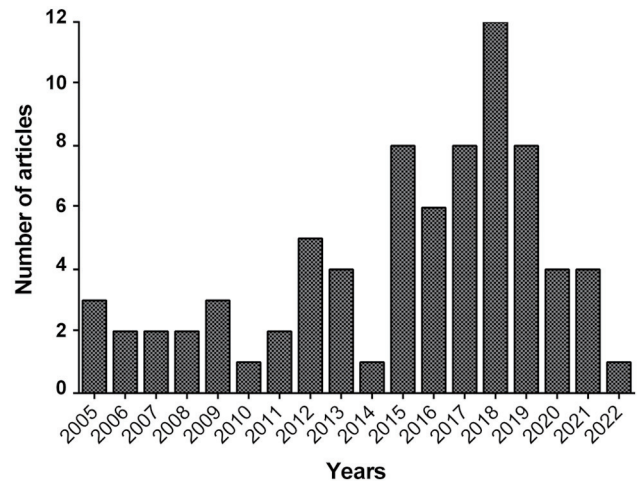
GSTP1 participates in the metabolism of drugs, such as oxaliplatin and adriamycin. In particular, in prostate cancer patients, the GSTP1 promoter is usually methylated and the methylation level is a marker for distinguishing either benign prostatic hyperplasia from prostate cancer or to predict the prognosis of prostate cancer or drug resistance.<sup>102</sup> In addition, the methylation level of NAT1 was detected to be higher (62%) in BC patients with tamoxifen-resistant tumors than in normal tissues.<sup>103</sup> These findings indicate that methylation of DMEs may contribute to drug resistance.

## DNA methylation and drug resistance in the female reproductive system

Deregulation of signaling pathways may occur through epigenetic changes being prominent in the onset of chemoresistance.<sup>49,104</sup> In the following section, the focus is on studies that analyzed DNA methylation associated with the development of drug-treated resistance. The histogram in Figure 6 shows the number of articles derived from PubMed that were specifically related to the investigation of samples from BC, OC, uterine cancer and cervical cancer patients from 2005 to 2022, which are discussed in the following paragraphs.

### Breast cancer

Estrogen receptor (ER)-positive BC is usually treated with tamoxifen, a drug that inhibits the binding of estrogen to its receptor; however, downregulation of ER $\alpha$  is the dominant mechanism of tamoxifen resistance.<sup>105</sup> Since the promoter region of ER is rich in CpG dinucleotides, the loss of expression of ER in tumors may be due to aberrant methylation of CpG islands. Epigenetic factors, such as DNMTs, histone deacetylases (HDACs), miRNAs and ubiquitin ligases are important regulators of ER loss in BC. Restoring the response to endocrine therapy through re-expression of ER $\alpha$  by inhibiting the expression of these regulators is, therefore, an essential component of a therapeutic approach.<sup>106</sup> The activation of DNMTs in BC was confirmed by Jahangiri *et al.*<sup>105</sup> For immunohistochemical experiments, they used 72 formalin-fixed



**Fig. 6. Histogram of the number of articles.** The number of articles specifically related to the investigation of samples from BC, OC, uterine cancer, and cervical cancer patients from 2005 to 2022 was used in this article.

paraffin-embedded (FFPE) tumor tissues from anti-estrogen tamoxifen sensitive and resistant BC patients. They demonstrated that DNMTs might be an effective factor in the development of tamoxifen resistance in BC.<sup>107</sup> In addition, they studied 107 BC tumors and normal breast tissues and revealed that the low methylation status of DNMT3A promoter and the overexpression of DNMT3B could contribute to disease recurrence in tamoxifen-treated BC patients.<sup>105</sup> Performing univariate and multivariate analysis, Xu *et al.*<sup>108</sup> compared cisplatin-resistant with cisplatin non-resistant triple-negative BC (TNBC) patients and demonstrated that cisplatin resistance was associated with ER $\alpha$  methylation. Therefore, ER $\alpha$  methylation might be a surrogate biomarker for outcome prediction and cisplatin resistance in TNBC patients. Not all ER-positive BC patients are responsive to endocrine therapy (*de novo* resistance). The resistance mechanism of ER-positive BC to neoadjuvant endocrine therapy was investigated by Jia *et al.*<sup>109</sup> A microarray was performed on 109 pairs of samples untreated and post-treated with neoadjuvant aromatase inhibitor therapy. Aromatase inhibitors, such as anastrozole, letrozole and exemestane, are alternatives to tamoxifen.<sup>110</sup> A study<sup>109</sup> found that the methylation of *BRCA2* led to incomplete suppression of RAD51, a key protein of homologous recombination;<sup>111</sup> therefore, causing an increased expression of RAD51 and then aromatase inhibitor resistance and poor prognosis in ER-positive BC patients. Selli *et al.*<sup>112</sup> investigated the long-term aromatase inhibitor-induced dormancy and acquired resistance in BC patients. In sequential tumor samples from BC patients receiving extended neoadjuvant aromatase inhibitor therapy, global loss of DNA methylation were observed in their tumors. Epigenetic alterations led to an escape from dormancy and drove acquired resistance in a subset of patients. The exemestane resistance was investigated by Liu *et al.*<sup>113</sup> They recruited 16 patients who received first-line exemestane-based hormone therapy and detected synchronized changes in methylation density and methylation ratio on chromosome 6 in the blood samples during exemestane treatment. They suggested that this DNA methylation may be a predictor of exemestane resistance.

Yu *et al.*<sup>114</sup> demonstrated that the protein levels of DNMTs correlated with the response to decitabine in patient-derived xenograft organoids derived from chemotherapy-sensitive and resistant TNBC patients. Depletion of TNF-associated factor 6, which, as an E3 ubiquitin ligase participates in the interleukin-1 receptor/



Toll-like receptor family and TNF receptor superfamily pathways,<sup>115</sup> blocked decitabine-induced DNMT degradation to confer resistance to decitabine.<sup>114</sup>

To date, methylation of the components of the cell cycle has been analyzed in relation to drug resistance.<sup>116</sup> In their genome-wide DNA methylation analysis, Klajic *et al.*<sup>117</sup> used paired tumor samples from locally advanced BC patients treated with doxorubicin and 5-fluorouracil-mitomycin C. They identified key cell cycle regulators differentially methylated before and after neoadjuvant chemotherapy, such as cyclin-dependent kinase (CDK) inhibitor 2A and *cyclin A1*. They suggested that the methylation patterns in these genes might be potential predictive markers of anthracycline or mitomycin sensitivity. The relevance of the CDK10 in the resistance to endocrine therapies was demonstrated by Iorns *et al.*<sup>118</sup> They reported that CDK10 silencing increased ETS2-driven transcription of *c-RAF*, resulting in activation of the MAPK pathway<sup>51</sup> and loss of tumor cell reliance upon ER signaling. Patients with ER $\alpha$ -positive tumors that expressed low levels of *CDK10*, because of promoter methylation, relapsed early on tamoxifen treatment.<sup>118</sup>

GSTP1 plays an important regulatory role in the detoxification by glutathione conjugation and anti-oxidative damage.<sup>119</sup> GSTP1 expression, along with the resistance to neoadjuvant paclitaxel followed by 5-fluorouracil/epirubicin/cyclophosphamide (P-FEC) in BC patients, was investigated by Miyake *et al.*<sup>120</sup> They detected that *GSTP1* expression could predict pathological response to P-FEC in ER-negative tumors but not in ER-positive tumors. However, *GSTP1* promoter hypermethylation might be implicated in the pathogenesis of luminal A, luminal B and human epidermal growth factor receptor 2 (HER2)-enriched tumors rather than basal-like tumors. Moreover, Arai *et al.*<sup>121</sup> suggested that GSTP1 protein expression, but not *GSTP1* methylation status, may be associated with the response to docetaxel and paclitaxel.<sup>121</sup>

Ye *et al.*<sup>122</sup> demonstrated that spalt-like transcription factor 2 (SALL2) that participates in growth arrest and pro-apoptotic functions,<sup>123</sup> upregulated *ER $\alpha$*  and *PTEN* through direct binding to their DNA promoters. However, its expression was significantly reduced during tamoxifen therapy in nine paired primary pretamoxifen-treated and relapsed tamoxifen-resistant BC tissues. Silencing of *SALL2* by hypermethylation induced downregulation of *ER $\alpha$*  and *PTEN* and activated the AKT/mTOR signaling pathway<sup>124</sup> resulting in ER-independent growth and tamoxifen resistance in ER $\alpha$ -positive BC. *In vivo* experiments showed that DNMT inhibitor-mediated *SALL2* restoration resensitized tamoxifen-resistant BC to tamoxifen therapy.<sup>125</sup>

Deregulation of steroid receptor coactivator (SRC) is especially involved in hormone-dependent tumors. By integrating steroid hormone signaling and growth factor pathways, SRC proteins exert diverse functions in oncogenic regulation in cancer.<sup>126</sup> Ward *et al.*<sup>127</sup> found that SRC-1 dependent epigenetic remodeling is a regulator of the poorly differentiated state in ER-positive BC. They revealed an epigenetic reprogramming pathway, where concerted differential DNA methylation was potentiated by SRC-1 in the endocrine-resistant setting. Jahangiri *et al.*<sup>128</sup> assessed *SRC-3* in 102 BC tissues and adjacent normal breast specimens. They observed overexpression of *SRC-3* combined with aberrant promoter methylation of the TF paired box 2 in tamoxifen-resistant BC patients compared with the sensitive ones.

Using Illumina Human Methylation Bead Chips (San Diego, CA, USA) for analyzing FFPE specimens, Gampenrieder *et al.*<sup>129</sup> performed genome-wide DNA methylation profiling of 36 HER2-negative metastatic BC patients under chemotherapy in combination with bevacizumab as first-line therapy. Significantly differentially methylated CpGs with an important change in methylation

levels between responders and non-responders were identified and further analyzed in 80 bevacizumab-treated BC patients and 15 patients treated with chemotherapy alone. A nine-gene methylation signature (*e.g.*, *WNT2B*, *MLH1*, *POLK*, *NOX4*, *PKNOX2*, *TMBIM6*, *SNRPN*, *UNC119*, and *GNAS*) and a three-gene signature (*e.g.*, *MLH1*, *POLK*, and *TMBIM6*) could discriminate between responders and non-responders to a bevacizumab-based therapy in metastatic BC patients.

Using a microarray-based technology, Martens *et al.*<sup>130</sup> examined the promoter methylation status of 117 candidate genes in a cohort of 200 steroid hormone receptor-positive tumors of patients who received tamoxifen as a first-line treatment for recurrent BC. They found that promoter hypermethylation and mRNA expression of phosphoserine aminotransferase (*PSAT1*) might act as indicators for a response to tamoxifen-based endocrine therapy in steroid hormone receptor-positive patients with recurrent BC.

Cancer patients have an elevated level of DNA in their blood, which is caused by active release (*e.g.*, apoptotic and necrotic cells) and active secretion (*i.e.*, extracellular vesicles).<sup>131,132</sup> The analyses of circulating methylated DNA in the blood of BC patients have been performed for drug resistance.<sup>133</sup> Measurements of serum DNA methylation were performed by Fiegl *et al.*<sup>134</sup> This laboratory showed that loss of Ras association domain family 1 isoform A (*RASSF1A*) DNA methylation in serum during treatment with tamoxifen highlighted a response, and the persistence or new appearance indicates resistance to adjuvant tamoxifen treatment.

### Ovarian cancer

Studies on pathways that contribute to the onset of chemoresistance in epithelial ovarian cancer (EOC) revealed hypermethylation-mediated repression of cell adhesion and tight junction pathways<sup>53</sup> and hypomethylation-mediated activation of the cell growth-promoting pathways<sup>135</sup> TGF-beta and cell cycle progression.<sup>136,137</sup>

Numerous studies reported that patients with platinum-resistant OC experienced poor outcomes.<sup>52</sup> In a clinical trial, tumors from primary high-grade serous OC (HGSOC) patients were compared with recurrent platinum-resistant HGSOC patients by Cardenas *et al.*<sup>138</sup> Differences in 452 CpG island-containing gene promoters that acquired DNA methylation in platinum-resistant and primary tumors were described. In primary platinum-resistant EOC patients, reduced representation of bisulfite sequencing was performed by Hua *et al.*<sup>139</sup> to screen for aberrantly methylated genes that might serve as potential epigenetic biomarkers for the prediction of primary platinum resistance. Nineteen differentially methylated regions located in the promoter region, which included *TRCGCA11-1*, *LOC105370912*, *ANO7P1*, *DHX4*, *MSH2*, *CDCP2*, *CCN1*, *ARHGAP42P2*, *PRDM13*, *LOC101928344*, *USP29*, *ZIC5*, *IL1RAPL1*, *EVX2*, *ABR*, *MGRN1*, *UBALD1*, *LINC00261*, and *ISL2*, were detected between eight primary platinum-resistant and eight extremely sensitive EOC patients. Furthermore, Yang *et al.*<sup>59</sup> suggested that 3-oxoacid CoA transferase 1 (*OXCT1*), a key enzyme in ketone body metabolism,<sup>140</sup> which was downregulated and hypermethylated at the promoter CpGs in cisplatin-resistant patients, might provide a potential therapeutic target for cisplatin chemotherapy in patients with recurrent EOC. Epigenetic inactivation of the putative DNA/RNA helicase Schlafen-11 (*SLFN11*) was identified as a predictor of resistance to platinum drugs in human cancer by Nogales *et al.*<sup>141</sup> EOC patients harboring hypermethylation of *SLFN11* had a poor response to cisplatin and carboplatin treatments. The CDK inhibitor *p57(Kip)2*, a cell cycle inhibitor,<sup>142</sup> is epigenetically regulated in carboplatin-resistant EOC patients.

Coley *et al.*<sup>143</sup> showed that silencing of *p57(Kip)2* decreased the apoptotic response under platinum treatment but produced sensitization to seliciclib. In addition, EOC biopsies indicated an association between high levels of *p57(Kip)2* mRNA with complete responses to chemotherapy and improved outcomes.

DNA damage repair pathways play an important role in supporting genomic integrity and DNA replication. Their dysfunction leads to accumulated DNA damage, predisposition to cancer and high sensitivity to chemotherapy and radiotherapy. Clinical studies suggest combining agents that target these pathways, such as poly (ADP-ribose) polymerase (PARP) inhibitors. No chemotherapy activates DNA damaging agents. Some types of chemotherapy cause DNA damage only for some drugs. Here, DNA mismatch repair (MMR) plays a role. Loss of MMR proteins lead to resistance in cancer patients, and there are emerging data that concern MMR deficiency in clinical drug resistance in EOC patients.<sup>144</sup> Its loss is accompanied by hypermethylation of the *hMLH1* gene promoter that occurs at a high frequency in EOC. Re-expression of *MLH1* is associated with a decrease in *hMLH1* gene promoter methylation.<sup>145,146</sup> Tian *et al.*<sup>60</sup> screened 16 platinum-sensitive or resistant samples from EOC patients with a reduced representation of bisulfite sequencing and detected that the upstream region of the *hMSH2* gene was hypermethylated in the platinum-resistant group.

Deregulation of cellular metabolism has been recognized as a key event in tumor growth and development, for example, argininosuccinate synthetase 1 (ASS1), which is a rate-limiting step in the arginine synthesis.<sup>147</sup> In EOC patients, *ASS1* methylation at diagnosis was associated with significantly reduced overall survival and relapse-free survival. In relapsed patients, *ASS1* methylation was significantly more frequent than in non-relapsed patients. These data, generated by Nicholson *et al.*<sup>148</sup> demonstrated the epigenetic inactivation of ASS1 as a factor of response to platinum chemotherapy and imply that transcriptional silencing of *ASS1* contributes to treatment failure and clinical relapse in EOC patients.

PLK2 is an acidophilic kinase belonging to the polo-like kinases (PLK), a family with five members with a central role in the cell cycle.<sup>149</sup> Syed *et al.*<sup>150</sup> reported that resistance might be conferred by the downregulation of PLK2. Experiments revealed that its downregulation occurred by DNA methylation of the CpG island in the *PLK2* gene promoter in primary tumors and serum of EOC patients. *PLK2* promoter methylation varied with the degree of drug resistance and transcriptional silencing of the promoter. In tumor tissues and matched sera, DNA methylation of the *PLK2* CpG island was associated with a higher risk of relapse in patients treated postoperatively with carboplatin and paclitaxel.

BRCA1 and BRCA2 participate in DNA repair processes and are important markers for BC and EOC. Apart from the hundreds of mutations identified in these genes, they are methylated. Their loss impairs DNA repair and causes irregularities in DNA synthesis.<sup>151</sup> In preclinical models and EOC patients, Kondrashova *et al.*<sup>152</sup> demonstrated that quantitative assessment of *BRCA1* methylation might provide information on the PARP inhibitor response. Analysis of 21 *BRCA1*-methylated platinum-sensitive recurrent HGSOE demonstrated that homozygous or hemizygous *BRCA1* methylation predicts rucaparib clinical response and that methylation loss can occur after exposure to chemotherapy.<sup>152</sup>

Homeobox (HOX) genes are developmental genes that code for TFs involved in embryogenesis. Numerous reports have shown that their altered expression can play key roles in the development of tumors.<sup>153</sup> Rusan *et al.*<sup>154</sup> revealed that *HOXA9* promoter methylation in circulating tumor DNA could serve as a biomarker in patients with platinum-resistant BRCA-mutated EOC undergoing treatment with PARP inhibitors. Bonito *et al.*<sup>155</sup> studied DNA methylation in independent tumor cohorts using Illumina Human Methylation ar-

rays. Hypomethylation of CpG sites within the Msh homeobox 1 (*MSX1*) gene was associated with resistant HGSOE disease and expression of *MSX1*, which resulted in platinum drug sensitivity.

High DNA methylation in normal 1 (HIN-1) was detected in paclitaxel-resistant tumor tissues of patients with ovarian clear cell carcinoma (OCCA) by Ho *et al.*<sup>156,157</sup> The demethylating agent 5-aza-2-deoxycytidine (5-aza-2-dC) reversed the methylation of *HIN-1*, reactivated the expression of *HIN-1*, to finally suppress the *in vivo* tumor growth of paclitaxel-resistant OCCA cells.<sup>156,157</sup> Li *et al.*<sup>158</sup> showed that methylation-associated *miR-9* downregulation might be responsible for paclitaxel resistance in EOC patients. Paclitaxel resistance is mediated by the deficiency of this miRNA that binds to *CCNG1*, a commonly induced p53 target.<sup>159</sup>

Chen *et al.*<sup>160</sup> examined the methylation of various genes in OCCA and ovarian endometrioid adenocarcinoma (OEA) and evaluated methylation biomarkers referring to patient chemo response and outcome. The frequencies of gene methylation in *RASSF1A* (79% versus 59%), a Ras effector that promotes the antiproliferative properties of Ras,<sup>161</sup> *E-cadherin* (30% versus 10%), a calcium-dependent, epithelial cell adhesion molecule<sup>162</sup> and deleted in lung and esophageal cancer 1 (*DLEC1*, 71% versus 43%)<sup>163</sup> were higher in OCCA patients than in OEA patients. The chemoresistant cohort had a higher percentage of *E-cadherin* methylation (36.7% versus 16.1%) than the chemosensitive group.<sup>160</sup>

In EOC, deficiency in human sulfatase-I (hSulf-1) is involved in the metabolic reprogramming of glycolysis and the cell cycle.<sup>164</sup> EOC patients who expressed higher levels of hSulf-1 displayed a 90% response rate to chemotherapy compared with a response rate of 63% in patients with weak or moderate levels. The findings reported by Staub *et al.*<sup>165</sup> indicated that *hSulf-1* was epigenetically silenced in EOC and that epigenetic therapy targeting *hSulf-1* might sensitize OC to conventional first-line therapies.<sup>165</sup>

Methylation controlled DNAJ (MCJ) is in the mitochondria.<sup>166</sup> Strathdee *et al.*<sup>167</sup> determined the methylation status of 35 CpG sites of an *MCJ* CpG island by sequencing sodium bisulfite modified tumor DNA derived from tumor tissues of 41 EOC patients at stage III/IV. The presence of high levels of CpG island methylation correlated significantly with poor response to therapy and poor overall survival.<sup>167</sup>

### Uterine cancer

Phosphoglycerate kinase 1 (PGK1) is a key glycolytic enzyme.<sup>168</sup> In endometrial cancer, Zhou *et al.*<sup>169</sup> reported that *PGK1* expression was elevated in tumor tissues and its high levels correlated with clinical stages and metastasis. PGK1 mediated DNA repair and methylation through the HSP90/ERK pathway, and eventually enhanced the chemoresistance to cisplatin. PGK1 interacted directly with the heat shock protein HSP90 and modulated the ATPase activity of HSP90, a molecular chaperone that assists in the conformational folding, stabilization and degradation of cellular proteins.<sup>170</sup>

### Cervical cancer

Septin 9 (SEPT9) is a member of the conserved family of cytoskeletal GTPases. It participates in numerous biological processes, such as cytokinesis, polarization, vesicle trafficking, membrane reconstruction, DNA repair, cell migration and apoptosis. For example, SEPT9 might serve as a marker for the early screening of colon cancer since the presence of freely circulating, methylated *SEPT9* DNA in blood plasma strongly correlates with the occurrence of colon cancer. The commercial SEPT9 test detects meth-

ylated DNA of the *SEPT9* gene in blood plasma to predict colon cancer.<sup>171</sup> Using methylation-specific PCR, Jiao *et al.*<sup>172</sup> detected methylated *SEPT9* in different cervical tissues. *SEPT9* promoted tumorigenesis and radioresistance in cervical cancer by targeting the high-mobility group box-1-retinoblastoma axis which participates in antitumor growth.<sup>173</sup> *SEPT9* was reported to be involved in proliferation, invasion, migration and influenced the cell cycle of cervical cancer.<sup>173</sup>

In total, 100 cervical cancer patients at FIGO stage IIB/III who underwent chemoradiation treatment were evaluated by Sood *et al.*<sup>174</sup> The methylation frequency of *ERα*, *BRCAl*, *RASSF1A*, *MLH1*, myogenic determination factor 1 (*MYOD1*) and human telomerase reverse transcriptase (*hTERT*) genes were from 40% to 70%. A pattern of unmethylated *MYOD1*, unmethylated *ERα*, methylated *hTERT* promoter, and lower *ERα* transcript levels predicted chemoradiation resistance.

Finally, Chaopatchayakul *et al.*<sup>175</sup> showed that aberrant DNA methylation of apoptotic signaling genes resulted in acquired resistance to therapy in cervical cancer patients. The methylation frequency of death-associated protein kinase and *FAS* molecules that play an important role in apoptosis,<sup>176</sup> exhibited a statistically significant difference between therapeutic non-responders and responders.<sup>175</sup>

### Epigenetic therapies

The main barrier to the successful treatment of cancer patients is the development of drug resistance. Therefore, the analysis of specific methylated biomarkers could improve cancer treatment and overcome drug resistance and recurrence. It is a high priority to understand these methylation changes that accompany cancer development and progression and therefore, be able to predict the patients that will benefit from specific treatment strategies. Epigenetic modifiers, such as DNMT inhibitors, in combination with HDAC inhibitors, have emerged as promising drug targets for cancer therapy in advanced-stage malignancies.<sup>177</sup> However, global genomic hypomethylation and acetylation might cause genomic instability, leading to chromosomal breaks.<sup>178</sup>

Thirty years ago, Jones and Taylor<sup>179</sup> reported that the analogs of cytidine, 5-aza-cytidine (5-aza-C) and 5-aza-2-dC induced differentiation of cultured mouse embryo cells to muscle cells. The ability of both drugs to induce differentiation and cell death provoked their investigation into the treatment of different cancer types. Both agents can be incorporated into DNA; however, 5-aza-C can be incorporated additionally into RNA and therefore, is an inhibitor for DNMT and RNA methyltransferases.<sup>180</sup> They have been demonstrated to be potent alternatives to conventional chemotherapy, particularly in the therapy of myelodysplastic syndrome and acute myeloid leukemia. Compared with conventional medical care, therapy of myelodysplastic syndrome with 5-aza-C doubled the 2-year survival rate of these patients.<sup>181</sup> The limitations of these components are their instability in aqueous solution, inactivation by cytidine deaminase to 5-azauridine and the potential re-establishment of DNA methylation by the withdrawal of DNMT inhibitors. However, another cytidine analog, zebularine, that lacks the amino group on C-4 of the pyrimidine ring is stable in an aqueous solution and can be administered orally.<sup>182</sup>

Histone deacetylase inhibitors have several functions. They modulate gene transcription by inhibiting the deacetylation of histones and proteins, including TFs. They inhibit proliferation at the G2 cell cycle checkpoint and upregulate pro-apoptotic molecules. In addition, they induce G1 cell cycle arrest via ac-

tivation of p21 in tumors with defective p53 function.<sup>183</sup> In several cancer types, histone deacetylase inhibitors are efficient in combination therapy, and cutaneous T-cell lymphoma was successfully treated by vorinostat alone that can be administered orally.<sup>184</sup> The development of drugs that target the epigenome (epi-drugs) to modulate the sensitivity of tumors to other anti-cancer drugs and to overcome therapy resistance continues and could provide new approaches to clinical investigations. To date, immunotherapy has emerged as an important strategy to treat cancer, because epigenetic processes are essential in regulating immune cell function and mediating antitumor immunity. A detailed report on these therapies was recently published by Topper *et al.*<sup>185</sup> Therefore, the development of epi-drugs should follow a precision-medicine approach with sequential treatment. A new generation of epi-drugs, which were developed for specific targets, have promising activity in populations with selected biomarkers. These have now entered early phase clinical trials and eventually might display promising efficacy.<sup>186</sup>

### Conclusions

In this review, DNA methylation related to gynecological tumors was discussed to gain a deeper insight into the epigenetic alterations that lead to the inactivation of tumor suppressors and DNA instability.<sup>187</sup> Epigenetic modifications can be investigated by numerous different techniques. As detailed in a review by Gouil *et al.*,<sup>188</sup> DNA methylation can be detected by bisulfite sequencing, methylation-specific PCR, multiplex ligation-dependent probe amplification, sequenom mass array technology, or methylation bead chip methodology.

However, to succeed in the detection of specific methylated biomarkers, a more genome-wide approach and screening methods must be applied. To date, investigations of the methylome have revealed important signaling pathways that contribute to therapy resistance, such as the Wnt<sup>189</sup> and PI3K/PTEN/AKT/mTOR<sup>124</sup> signaling pathways and cell adhesion or tight junction pathways.<sup>53</sup> The deregulation of cellular metabolism and DNA damage repair are examples of DNA hypermethylation.<sup>144</sup> Some methylation patterns have been established for specific tumors; however, few DNA methylation patterns have the specificity and sensitivity to identify specific cancer types with certainty. Despite these shortcomings, regimens with demethylating agents combined with standard therapies appear to be encouraging. Studies have attempted to change drug resistance-associated DNA methylation patterns using DNMT- and TET-dependent demethylation methods.<sup>190</sup> These agents provided an imbalance in the global DNA methylation pattern that caused the activation of tumor suppressor genes and oncogenes, which resulted in undesirable side effects. Therefore, a fine balance between DNA methylation is necessary to establish a correct drug response.

Future experiments will determine whether interventions into the methylation patterns will succeed in overcoming drug resistance.

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**Conflict of interest**

The authors have no conflicts of interest related to this publication.

**Author contributions**

HS wrote the paragraph “DNA methylation and drug resistance in the female reproductive system” and “Epigenetic therapies”, and created the figures and the table. PBG wrote the introduction and “DNA methylation and drug resistance in cancer cells” and checked the English. HS and PBG wrote together the paragraphs on signaling pathways, drug transport and metabolism, and the conclusion.

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