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## Review

## Epigenetic regulation of DNA repair genes and implications for tumor therapy

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## ABSTRACT

DNA repair represents the first barrier against genotoxic stress causing metabolic changes, inflammation and cancer. Besides its role in preventing cancer, DNA repair needs also to be considered during cancer treatment with radiation and DNA damaging drugs as it impacts therapy outcome. The DNA repair capacity is mainly governed by the expression level of repair genes. Alterations in the expression of repair genes can occur due to mutations in their coding or promoter region, changes in the expression of transcription factors activating or repressing these genes, and/or epigenetic factors changing histone modifications and CpG promoter methylation or demethylation levels. In this review we provide an overview on the epigenetic regulation of DNA repair genes. We summarize the mechanisms underlying CpG methylation and demethylation, with *de novo* methyltransferases and DNA repair involved in gain and loss of CpG methylation, respectively. We discuss the role of components of the DNA damage response, p53, PARP-1 and GADD45a on the regulation of the DNA (cytosine-5)-methyltransferase DNMT1, the key enzyme responsible for gene silencing. We stress the relevance of epigenetic silencing of DNA repair genes for tumor formation and tumor therapy. A paradigmatic example is provided by the DNA repair protein *O*<sup>6</sup>-methylguanine-DNA methyltransferase (MGMT), which is silenced in up to 40% of various cancers through CpG promoter methylation. The CpG methylation status of the MGMT promoter strongly correlates with clinical outcome and, therefore, is used as prognostic marker during glioblastoma therapy. Mismatch repair genes are also subject of epigenetic silencing, which was shown to correlate with colorectal cancer formation. For many other repair genes shown to be epigenetically regulated the clinical outcome is not yet clear. We also address the question of whether genotoxic stress itself can lead to epigenetic alterations of genes encoding proteins involved in the defense against genotoxic stress.

## 1. Introduction

Genotoxic agents cause cell death or, in the survivors, mutations and metabolic changes that can finally lead to cancer. To counteract these disastrous effects, cells are equipped with DNA repair mechanisms that either remove or tolerate the damage. These repair and tolerance mechanisms contribute to the genomic integrity of the cell. More than 200 DNA repair proteins have been identified and their role in DNA repair has been elucidated [1,2]. The expression of DNA repair proteins is regulated on multiple levels. Transcriptional regulation is dependent on the availability and activation of transcription factors that guarantee a quick response to exogenous and endogenously generated genotoxins. Besides the regulation on this level, which was reviewed previously [3], DNA repair genes are also regulated epigenetically. A compilation of DNA repair genes shown to be subject of epigenetic regulation is given in Table 1. Whereas transcriptional regulation causes transient alteration of gene expression, epigenetic regulation usually results in

sustained or even permanent changes. These are, as a rule, associated with complete silencing of gene expression, which is commonly observed in tumours. Similar to mutations, epigenetic silencing of DNA repair factors is often causally related to tumorigenesis. However, in contrast to mutations, epigenetic alterations do not change the underlying DNA sequence [4], which means that epigenetic alterations are reversible and, therefore, targetable. Epigenetic regulation of gene expression is governed by controlling the access of proteins, notably transcription factors, to their target gene. This is accomplished through compacting (condensation) or opening up (de-condensation) of the chromatin [5]. The two most prominent processes provoking changes in chromatin compaction rest on histone modification and DNA methylation at the 5-position of cytosine.

## 2. Histone modification

Transient and stable silencing of promoters can be accomplished by

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**Table 1**  
DNA repair genes regulated on epigenetic level.

Gene	Cancer type	Frequency	Ref.
<b>atm</b>	Breast Cancer	78%	[154]
	Head and Neck Squamous Cell Carcinoma	25%	[151]
	Non Small Cell Lung Cancer	19–47%	[152]
<b>alkbh3</b>	Breast cancer	27%	[65]
<b>brca1</b>	Breast Cancer	11–38%	[45,101,113–115]
	Ovarian Cancer	5–19%	[45,114–116,120]
<b>brca2</b>	Gastric Cancer	60%	[211]
	Non Small Cell Lung Cancer	4–30%	[45,97,120,212]
	Uterine Leiomyosarcoma	25%	[121]
	Bladder Cancer	12.1%	[122]
	Breast Cancer	57–69.2%	[141,142]
<b>chk2</b>	Ductal Carcinoma <i>in situ</i>	59%	[143]
	Invasive Ductal Cancer	64%	[143]
	Non Small Cell Lung Cancer	42%	[120]
<b>ercc1</b>	Laryngeal Squamous Cell Carcinoma	46%	[123]
<b>fancC</b>	Non Small Cell Lung Cancer	28%	[157]
	Glioma	37.5%	[86]
<b>fancF</b>	Sporadic Leukemia	2.5%	[105]
	Head and Neck Squamous Cell Carcinoma	42%	[106]
<b>fancF</b>	Cervical Cancer	30%	[96]
	Non Small Cell Lung Cancer	14%	[97]
	Ovarian Cancer	13–28%	[100]/[98]/[99]
<b>fen1</b>	Head and Neck Squamous Cell Carcinoma	15%	[97]
	Sporadic breast cancer	1–4%	[101]/[102]
	Bladder cancer	Single case (1/41)	[103]
	Breast Cancer	33.3%	[132]
	Non Small Cell Lung Cancer	20%	[120]
<b>ku80</b>	Colorectal Cancer	24%	[127]
<b>mbd4</b>	Various brain cancers	38%	[41]
	Glioblastoma	34–45%	[41,45–49]
<b>mgmt</b>	Intrahepatic Cholangiocarcinomas	11%	[213]
	Non-Seminomas/Seminomas	69%/24%	[214]
<b>mlh1</b>	Cervix Cancer	26–29%	[215]
	Bladder Cancer	2%	[216]
	Head and Neck Squamous Cell Carcinoma	28%/53%	[41]/[217]
	Lymphomas	25%	[41]
	Colorectal Cancer	26%	[41]
	Lung tumors	24%	[41]
	Pancreatic Cancer	11%	[41]
	Melanoma	11%	[41]
	Renal Carcinoma	8%	[41]
	Leukemia	6%	[41]
	Non Small Cell Lung Cancer	8–50%	[152,218–221]
	Astrocytoma	15%	[76]
	HNPC	Single Case	[222,223]
	Acute Myeloid Leukemia	2/55	[224]
	Gastric Cancer	9–32%	[69,73,84,225,226]
	Head and Neck Squamous Cell Carcinoma	69%	[217]
	Non Small Cell Lung Cancer	56%	[78]
	Oral squamous cell cancer	17–47%	[79–81,227]
	Ovarian Cancer	12.5%/30.4%	[228]/[82]
	Sporadic Colorectal Cancer	38%	[70]
	Sporadic Endometrial Carcinoma	45/41%	[71] [72]
Epithelial Ovarian Cancer	56.3	[75]	
T-cell leukemia/lymphoma	6%	[229].	
Head and Neck Squamous Cell Carcinoma	28.6%	[230]	
Gastric Cancer	21.6%	[231]	
Oral Squamous Cell Carcinoma	76%	[232]	
Esophageal Squamous Cell Carcinoma	23%	[233]	
Pancreatic Cancer	23%	[234]	
Ovarian Cancer	37.5%	[235]	
Non Small Cell Lung Cancer	31.3%	[236]	
		2–68%	[78,152,219,237,238]

**Table 1 (continued)**

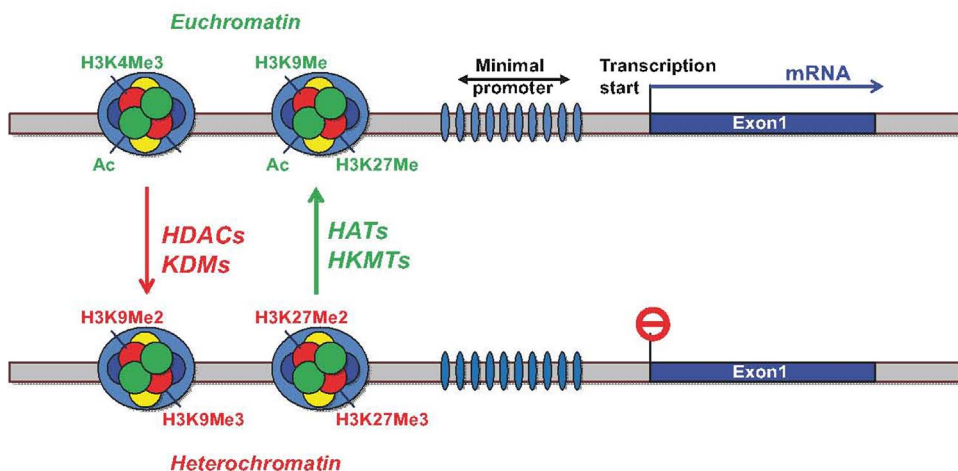
Gene	Cancer type	Frequency	Ref.
<b>mpg</b>	Glioblastoma	50%	[128]
<b>msh2</b>	Non Small Cell Lung Cancer	28.6%	[78]
	Oral Squamous Cell Carcinoma	27–36%	[79–81]
	Ovarian Cancer	51.7%	[82]
	Colorectal Cancer (MSH2-deficient)	24%	[77]
<b>msh3</b>	Gastric Cancer	15.6%	[84]
	Gastric cancer	14.3%	[84]
<b>ogg1</b>	Thyroid Cancer	5%	[129]
<b>wrr</b>	colorectal cancer (37.9%); non-small cell lung cancer (37.5%); chondrosarcomas (33.3%); gastric cancer (25%); non-Hodgkin lymphoma (23.7%); prostate cancer (20%); breast cancer (17.2%); thyroid tumors (12.5%); osteosarcomas (11.1%); acute lymphoblastic leukemia (9.5%); acute myeloblastic leukemia (4.8%)		[146]
	Oral Squamous Cell Carcinoma	23.8%	[147]
<b>xpc</b>	Bladder Cancer	32.4%	[91]
	Non Small Cell Lung Cancer	33.5%	[90]
<b>xrcc1</b>	Gastric cancer	76,4	[134]

numerous covalent histone modifications, such as acetylation, methylation, phosphorylation and ubiquitination at the N-terminal tails [6]. For example, acetylation of N-terminal lysine residues of histones H3 and H4 is associated with active chromatin, while methylation of lysine 9 and 27 of histone H3 appears to be the hallmark of condensed chromatin at silent loci [7–9] (Fig. 1A). Histone modifications exert their effects via direct or indirect influencing the overall structure of chromatin. Histone acetylation and phosphorylation reduce the positive charge of histones, thus disrupting the electrostatic interaction of proteins with the DNA [10]. Opposed to this, methylation does not provoke alterations in the overall charge of histones but regulates binding of effector molecules like chromatin remodelers or transcription factors [11]. Histone modifications are regulated by the action of opposing enzyme pairs (Fig. 1A). Acetylation of lysine is regulated by histone acetyltransferases (HATs) and deacetylases (HDACs). Since histone acetylation leads to opening of the chromatin, HDACs act predominantly as transcriptional repressors. Methylation occurs predominantly at lysine residues of histones, which can be mono-, di- or tri-methylated by histone lysine methyltransferase (HKMT) with S-adenosylmethionine (SAM) as methyl group donor. Demethylation is mediated by different histone Lys demethylases (KDMs), belonging to either the amino oxidase or the JmjC family. Generally, acetylation/deacetylation of histones leads to transient alterations in gene expression, whereas methylation/demethylation can either lead to activation or facultative and constitutive silencing, depending on the histone, the residue and the type of modification. For stable gene silencing, histone modifications cross-talk with mechanisms leading to methylation of the promoter. Besides regulating chromatin structure HDACs are also involved in regulating the activity of various DNA repair systems [12].

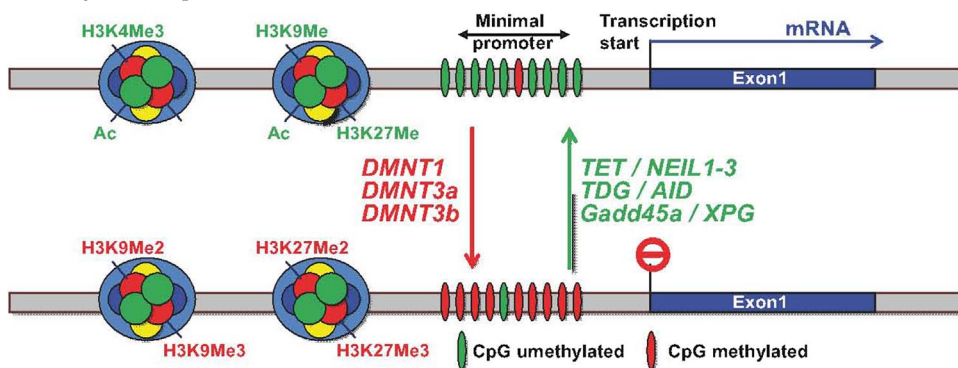
### 3. DNA methylation

Enzymatic DNA methylation occurs at the 5-C position of cytosine (5-methylcytosine, 5meC) in hemi methylated CpG di-nucleotides, so called CpG islands. CpG islands are 0.5 – 2 kB regions that are located in the 5' promoter regions of up to 50% of all human genes [13]. Methylation of CpG islands is frequently preceded by histone modifications and rearrangements of the chromosome structure, which finally leads to methylation of CpG in the transcription factor binding site area (briefly named promoter methylation) resulting in transcriptional silencing of the gene (Fig. 1B). Centromeric and pericentromeric regions as well as other repetitive elements are strongly methylated, and also many areas

## A Histone modification



## B CpG methylation



**Fig. 1.** Mechanisms of epigenetic gene regulation. A) Histone modification is regulated by opposing enzymes. Acetylation of lysine is mediated by histone acetyltransferases (HATs) and deacetylation by histone deacetylases (HDACs). Methylation is mediated by histone lysine methyltransferase (HKMTs) and demethylation by histone lysine demethylases. B) DNA methylation at 5-methylcytosine (5mC) is performed by DNA (cytosine-5)-methyltransferases (DNMT1, DNMT3A and DNMT3B) whereas demethylation is accomplished by TET methylcytosine dioxygenases (TET1-3), the activation-induced deaminase (AID), Gadd45a/XPG, and the glycosylases TDG, UNG, NEIL1/2 3.

in the coding region of genes show a high degree of methylation. On contrary to this, CpG islands which are located in the promoter region are largely unmethylated. This methylation pattern, once established is normally maintained throughout cell division. However, alteration of the epigenetic profile can be accomplished by targeted *de novo* methylation and demethylation [14].

Cytosine methylation is performed by DNA (cytosine-5)-methyltransferases [15]. While DNMT1 functions as a maintenance methyltransferase to methylate cytosine in the newly synthesized DNA strand immediately after replication, DNMT3A and DNMT3 B methylate *de novo* cytosine [16] (Fig. 1B). A third member of the DNMT3 family, DNMT3L, acts as a regulator of DNMT3A and DNMT3 B [17]. Transcriptional repression by methyl-CpG is mediated by additional methyl-DNA binding proteins (MBD1, MBD2, MBD3, MeCP2 and MBD4) or zinc finger domain proteins (Kaiso, ZBTB4 and ZBTB38)[18]. These proteins can further recruit chromatin remodeling factors such as histone deacetylases and histone H3 lysine 9 methylases [19].

## 4. DNA demethylation

Whereas the mechanism of DNA methylation is well understood, the mechanism underlying active demethylation remained enigmatic for long time. A conceivable way to demethylate the DNA would be an enzymatic removal of the methyl group from the 5-position of cytosine. This mechanism however is highly unlikely to occur due to the high strength of the carbon-carbon bond at the 5-position of cytosine. As an alternative strategy, a cut-out process can be considered. This is what nature does. The detailed mechanism was resolved quite

recently. It was shown that the removal of 5-methylcytosine rests on the enzymatic modification of 5-methylcytosine by the activation-induced deaminase (AID) and the TET methylcytosine dioxygenases followed by base excision repair see (Fig. 2). In more detail, the mechanism involves enzymatic deamination of 5-methylcytosine to thymine by AID, followed by BER or MMR that replace thymine with cytosine [20]. A second putative mechanism seems to implicate the function of Gadd45a and the NER endonuclease XPG [21]. In this case, it was shown that ectopic expression of Gadd45a correlates with a partial reduction in methylation at the Oct4 promoter in *Xenopus laevis* oocytes. However, using human Gadd45a instead of frog Gadd45a, demethylation could not be achieved [22]. Therefore, it is conceivable that the possible involvement of Gadd45a and XPG in the demethylation process is cell specific and presumably reflects an unknown role in excision repair [23,24].

Most recently it was shown that 5-methylcytosine demethylation involves the base 5-hydroxymethylcytosine (5hmC), which was described as the sixth base of the mammalian genome [25]. 5hmC is generated from 5mC via a Fe(II) and alpha-ketoglutarate ( $\alpha$ -KG)-dependent oxidation reaction [26,27]. The reaction is mediated by the TET (ten eleven translocation) methylcytosine dioxygenases (TET1-3), which also catalyzes the conversion of 5hmC to 5-formylcytosine (5fC) and further to 5-carboxylcytosine (5caC). 5fC and 5caC are substrates for the TDG and therefore can be repaired by BER [28]. An alternative pathway involves deamination of 5hmC to 5-hydroxymethyluracil (5hmU) by AID, followed by SMUG1, UNG and TDG mediated BER [29]. In addition, the glycosylases NEIL1, 2 and 3 can partially substitute TDG in this process [30,31] (Fig. 2).

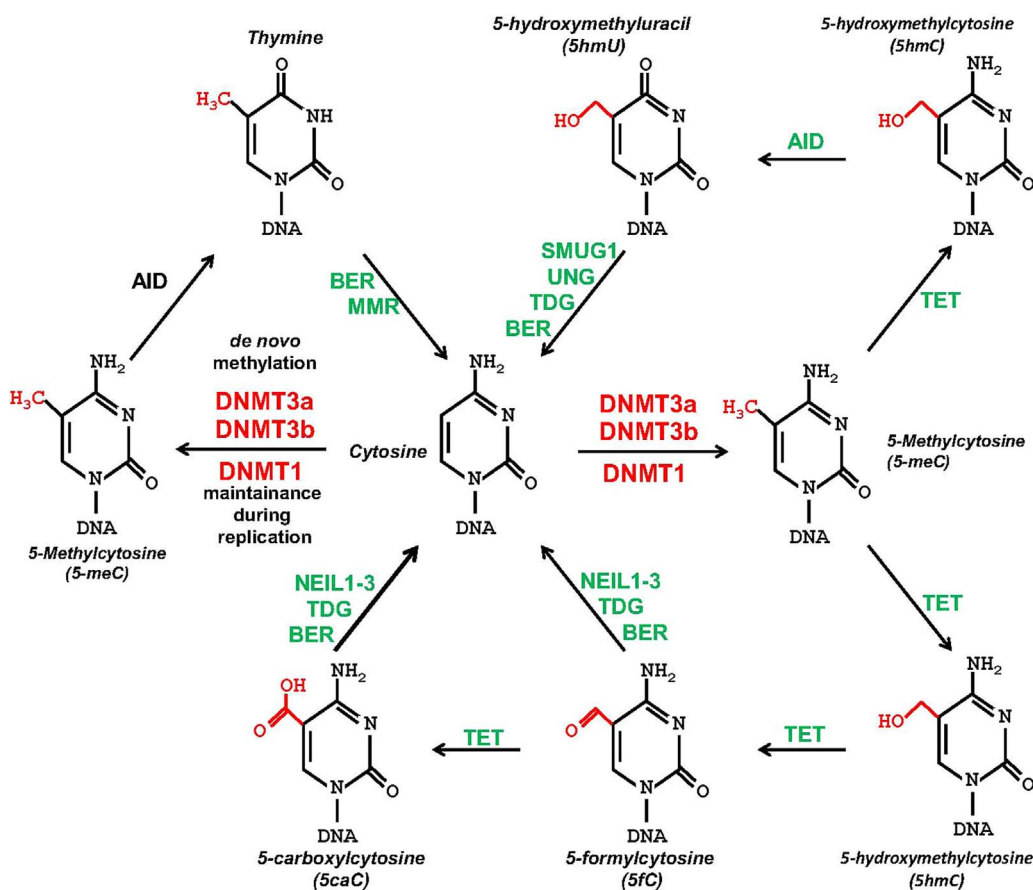


Fig. 2. Metabolism of 5-methylcytosine.

## 5. Regulation of DNA repair genes by promoter methylation

### 5.1. Epigenetic regulation of *MGMT*

Regarding epigenetic regulation of genes, the most intensively studied DNA repair gene is the alkyltransferase *MGMT* [32,33]. As many as 97 CpG islands have been identified in the *MGMT* promoter and methylation of two CpG clusters positioned between  $-249$  and  $-103$  and between  $+107$  and  $+196$  have been shown to provoke transcriptional silencing [34–36]. Under non-methylated conditions, the transcription start site is flanked by four precisely positioned nucleosome-like structures (Fig. 3A). Methylation of the CpG-islands results in heterochromatinization, which is accomplished by the re-arrangement of these nucleosomes, which now shield the transcription start site from the transcription machinery [32,37,38] (Fig. 3B). As expected, methylation of the CpG-islands within the *MGMT* promoter strongly correlates with *MGMT* activity (Fig. 3C). Besides methylation of the promoter, methylation within the body of the gene has been shown to impact on *MGMT* expression. In this case, methylation in the exon-containing regions resulted in up-regulation of *MGMT* expression [39], which was accompanied by acquired resistance of melanoma cells to the anticancer drug fotemustine [40].

Inactivation of *MGMT* by promoter hypermethylation is a common event in various primary human cancers like glioma, head and neck cancer, lymphoma, colorectal and lung tumors, pancreatic carcinoma, melanoma, renal carcinoma, leukemia and bladder carcinoma [41]. It was not at all observed in breast cancer, ovarian cancer and endometrial cancer (for review see [42–44]). Most analyses were conducted in glioblastoma, showing epigenetic silencing of *MGMT* in 34–45% of the cases [41,45–49]. *MGMT* promoter methylation was also observed in up to 33% of recurrent glioma [49–51].

As *MGMT* is the main determinant of the cell's sensitivity to

methylation and chloroethylating anticancer drugs [52,53], its expression level [44] (Fig. 3D) as well as the methylation status of the *MGMT* promoter (Fig. 3E) correlate with the survival of glioblastoma patients treated with these drugs. Promoter methylation of *MGMT* is therefore used as a prognostic marker for the therapeutic response of glioblastoma patients treated with alkylating agents, i.e. the methylating drug temozolomide and the chloroethylating agent lomustine (CCNU) or nimustine (ACNU) [44].

Similar to glioblastoma patients, also melanoma patients in a metastatic case, notably with brain metastases, undergo treatment with alkylating agents. Overall, *MGMT* methylation was detected in 29.7% [54], 31% [55] and 34% [56] of metastatic malignant melanoma. In disseminated cutaneous melanoma [57] and metastatic melanoma [58] an association between *MGMT* promoter methylation and the response to TMZ treatment was observed, and *MGMT* gene promoter methylation was associated with longer survival of patients suffering from metastatic melanoma, irrespective of therapy [54].

*MGMT* promoter methylation also represents a frequent event in the development of colorectal cancer [59]. In this type of cancer, no association with therapy response was observed. Of note, these tumors are not subject of treatment with alkylating agents, which might explain this lack of correlation. In detail, *MGMT* promoter hypermethylation was observed in 38% of 855 colon and rectal cancers, and no association with patient survival was observed [60]. In addition, *MGMT* promoter methylation was detected in 64.2% of brain metastases from colorectal cancer, and the therapeutic outcome was again independent of the *MGMT* status [61]. A recent meta-analysis showed that *MGMT* promoter methylation is central to the development of colorectal cancer, but is not associated with the prognosis [62]. *MGMT* promoter methylation was further observed in brain metastases derived from lung cancer (46.5%), breast cancer (28.8%), malignant melanoma (24.7%) and renal cancer (20%) [63], as well as in several other cancer entities (Table 1).

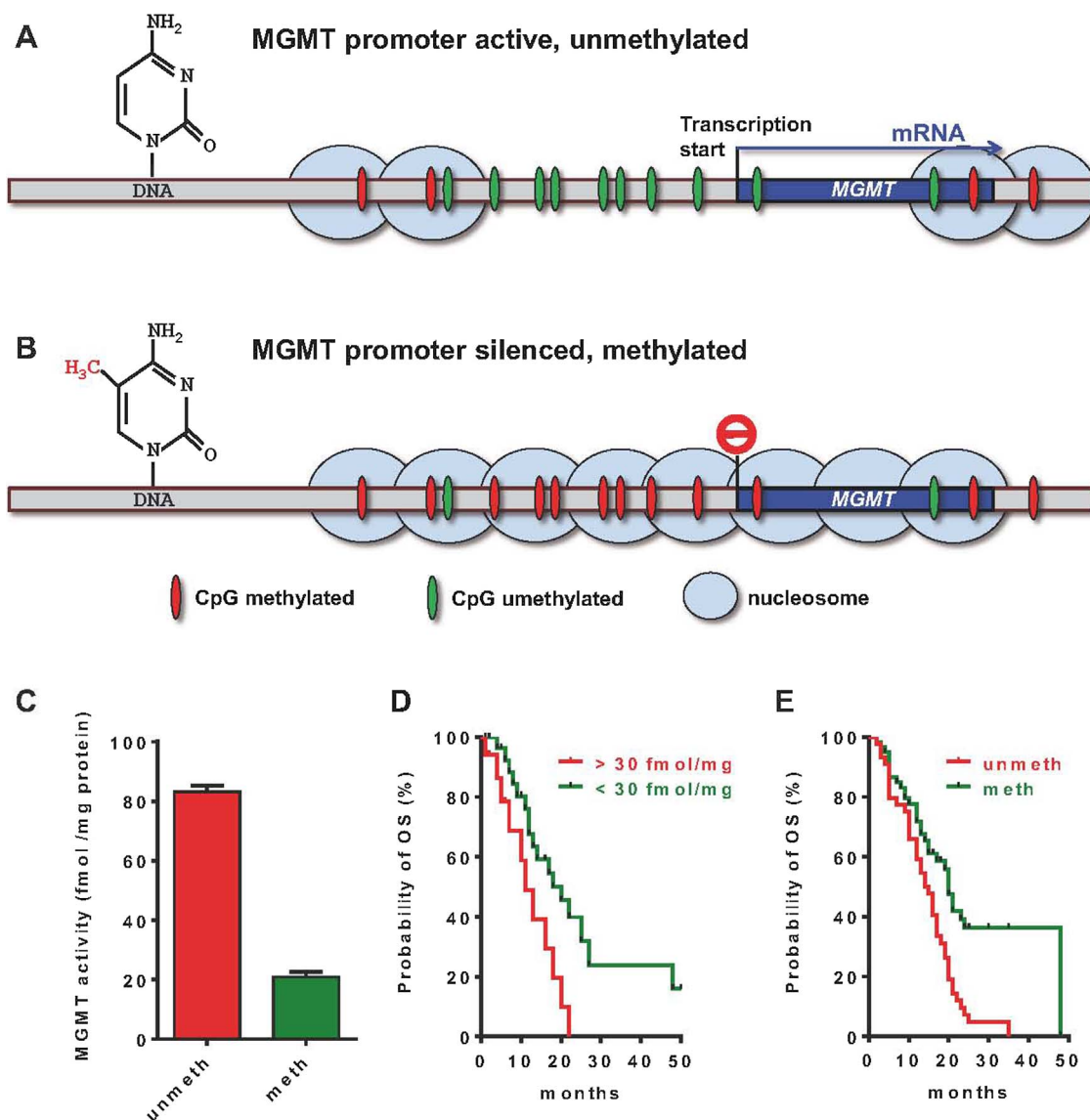


Fig. 3. Promoter methylation of MGMT. Within the *MGMT* promoter, the transcription start site is flanked by four nucleosome-like structures allowing transcription. Upon methylation of two CpG clusters positioned between  $-249$  and  $-103$  and between  $+107$  and  $+196$  re-arrangement of these nucleosomes occurs, shield the transcription start site and preventing transcription.

In addition to MGMT, the  $\alpha$ -ketoglutarate dependent dioxygenases ALKBH2 and ALKBH3 (repair enzymes homologous of *E. coli* ALKB protein) are involved in the direct reversal of alkylation damage. Thus, ALKBH2 and ALKBH3 catalyzes the removal of 1-methyladenine and 3-methylcytosine, which are both supposed to be cytotoxic lesions [64]. In a recent study it was shown that the human ALKBH3 promoter is methylated in 27% of the cases, which was associated with reduced survival of breast cancer patients [65].

## 5.2. Epigenetic regulation of mismatch repair (MMR)

MMR is responsible for the detection and repair of single base mismatches and small insertion/deletion mismatches generated during replication. During repair the mismatch is recognized by the MutS $\alpha$  complex (MSH2 and MSH6). Upon interaction with MutL $\alpha$  (MLH1 and PMS2), the complex translocates along the DNA in search for pre-existing SSBs. These SSBs arise at the end of the Okazaki fragments or result from RNaseH mediated removal of uracil in the newly synthesized DNA strand. Starting from this SSB, the exonuclease EXO1, together with the MutS $\alpha$  and MutL $\alpha$  complex translocates back, thereby

removing the newly synthesized DNA containing the mismatch. Defects in MMR lead to microsatellite instability that is causally involved in the development of hereditary non-polyposis colon cancer (HNPCC, Lynch-Syndrome) and in several sporadic developing cancer types [66,67].

Methylation of the *MLH1* promoter has first been observed in HNPCC and it has been shown that methylation in the promoter region of *MLH1* correlates with decreased activity of the gene [68]. Methylation of the *MLH1* promoter highly correlates with microsatellite instability (MSI). Thus, *MLH1* promoter methylation was found nearly exclusively in MSI+ tumors compared to MSI- cancers from the same group [69-73]. In contrast to MGMT, the *MLH1* promoter methylation status is not yet being used as a prognostic marker. It could be that hypermethylation is a common event in the formation of colon cancer. In this case the so-called CpG island methylator phenotype (CIMP) is being observed, which is marked by methylation of multiple promoter CpG islands. Interestingly, in a large study analyzing 649 colon cancers, patients with tumors harboring at least 6 out of 8 methylated CIMP-specific promoters (amongst them the *MLH1* promoter) showed a lower colon cancer specific mortality [74].

*MLH1* promoter methylation was also observed in advanced

epithelial ovarian cancer, however only recurrences, but not primary tumors showed promoter methylation [75]. In this case, methylation of the *MLH1* promoter was associated with acquired resistance to cisplatin and carboplatin [75]. Concerning the response to anticancer therapy, a second report for astrocytoma is available. Here, promoter methylation of *MLH1* was associated with longer progression-free survival upon treatment with ACNU [76]. Methylation of the *MLH1* promoter was also observed in head and neck squamous cell carcinoma, esophageal squamous cell carcinoma, oral squamous cell carcinoma, NSCLC, gastric cancer, pancreatic cancer, endometrial carcinoma and leukemia (Table 1).

In comparison to *MLH1*, *MSH2* appears to be less intensively regulated by promoter methylation since several studies, in which *MLH1* and *MSH2* promoter methylation was analysed, were negative as to methylation of the *MSH2* promoter. Nevertheless, *MSH2* promoter methylation was observed in colorectal cancer [77], NSCLC [78], oral squamous cell cancer (OSCC) [79–81] and ovarian cancer [82]. Interestingly, *MSH2* was also found to be methylated in 33% of neurofibromatosis type 1 patients [83]. *MSH3* represents an additional factor in MMR. Together with *MSH2* it forms the heterodimer MutS $\beta$ , which corrects long insertion/deletion loops microsatellites during DNA synthesis. The *MSH3* promoter was found to be methylated and epigenetically inactivated in gastric carcinoma [84].

### 5.3. Epigenetic regulation of nucleotide excision repair (NER)

Similar to MMR, a deficiency in NER gene expression is associated with genomic instability syndrome, leading to enhanced cancer incidence. Thus mutations in NER genes result in the well-known hereditary DNA repair defects *xeroderma pigmentosum*, *Cockayne's syndrome* and *trichothiodystrophy*. Interestingly, epigenetic silencing of NER genes was found to be associated with hyper-sensitivity to anticancer drugs. NER consists of two distinct pathways, namely global genomic repair (GGR) and transcription-coupled repair (TCR). During GGR, the DNA damage is recognized by the XPC–HR23 B and DDB1–DDB2 complexes and further verified by XPA. TFIIH containing the two DNA helicases XPB and XPD unwinds the DNA strand and the damaged DNA strand is incised by the endonucleases ERCC1–XPF and XPG. Upon removal of the damaged DNA region, DNA synthesis and ligation occurs. During TCR the DNA lesion is detected via blockage of RNAPII, leading to assembly of CSA and CSB at the site of the lesion. Upon back-tracking or removal of RNAPII CSB interacts with XPG, initiating DNA repair similar to GGR.

Epigenetic silencing of NER genes has been reported only in few reports. Thus, a study described methylation of *XPG* in murine cells and further detected *XPG* methylation in 5 out of 26 human ovarian cancer [85]. There are also reports describing epigenetic silencing of *ERCC1*, *hRAD23b* and *XPC*. The *ERCC1* promoter comprises a region between nucleotides –5503 and –5280. In this region, CpG hypermethylation was observed in 3 out of 5 glioma cell lines and in 12 out of 32 of glioma tumor samples [86]. Moreover, it was shown that methylation of the *ERCC1* promoter inversely correlates with the expression of *ERCC1* mRNA, ERCC1 protein and cisplatin sensitivity. In glioma cell lines *ERCC1* promoter methylation was also associated with radiosensitivity [87]. The *hRad23b* gene contains 47 CpGs and CpAs between nucleotides –338 and –64. Methylation of these sites was reported in interleukin-6 responsive myeloma KAS-6/1 cells. In these experiments promoter methylation correlated with gene expression [88]. Only limited data is available for the presence of these methylation in human cancers. Thus infrequent methylation (2%) in *ERCC1* and *hRad23b* has been described in non-small cell lung cancer [89]. The *XPC* promoter contains 17 CpG islands between nucleotides –175 and –1 and hypermethylation of this region correlates with *XPC* expression in lung cancer cell lines [90]. *XPC* hypermethylation was further detected in 33.5% of lung cancers where it was predominantly found in non-smokers (41%) compared to smokers (22%) and was also observed in

bladder cancer tissue [91]. Concerning *CSB* (*ERCC6*), hypermethylation of one CpG site was found to be associated with gene repression in the lens tissue from patients suffering from age-related nuclear cataract [92].

### 5.4. Epigenetic regulation of interstrand crosslink repair

DNA interstrand cross-links (ICLs) covalently connect the DNA strands and thus lead to blockage of DNA replication and transcription and finally cell death. Due to the high toxicity of this process, anticancer drugs inducing ICLs, like cisplatin, are widely used. ICLs are repaired by the combined action of various DNA repair proteins belonging to different repair mechanisms such as NER, homologous recombination and translesion synthesis. Important components of ICL repair, that detect and coordinate these mechanisms, are *Fanconi anemia* (FANC) proteins. Deficiency in either one of these 15 proteins results in *Fanconi anemia*, a genomic instability disorder with high incidence of cancer. In the Fanconi pathway eight FANC proteins (FANCA, B, C, E, F, G, L, M) form a complex that detects ICL and subsequently mono-ubiquitinates two other FANC proteins (FANCD2 and FANCI), leading to their activation. FANCD2 interacts with the FANCN/FANCD1 (BRCA2) complex and thereby regulates homologous recombination. In addition, FANCD2 interacts with BRCA1, which in turn can interact with the helicase FANCI, thereby enhancing homologous recombination and inhibiting translesion synthesis [93], or with the nuclease FANL1, which is involved in the incision/excision step.

Initially it has been shown that the *FANCF* promoter contains 61 potential methylation sites within the nucleotides –330 to +440 [94]. This region was hypermethylated in 4 out of 25 ovarian cancer cell lines and in 4 out of 19 ovary tumors, and hypermethylation was shown to be associated with sensitivity to cisplatin [94]. In addition, hypermethylation was observed in 36 out of 112 epithelial ovarian cancers, which was associated with prognosis [95], as well as in cervical, [96], lung [97] and ovarian cancer [98–100], and at very low frequency in sporadic breast [101,102] and bladder cancer [103]. Experimentally, hypermethylation of *FANCF* has been reported in SiHa cells after treatment with curcumin, resulting in a fivefold upregulation of gene expression [104]. Hypermethylation of the *FANCC* promoter was observed in 1/143 AML bone marrow samples and 3/97 acute lymphoblastic leukemia (ALL) samples, which was correlated with increased sensitivity to mitomycin C [105]. Hypermethylation of the *FANCC* promoter was also reported in head and neck cancer [106]. Furthermore also hypermethylation of the *FANCL* promoter was observed in 1/97 ALL samples [105]. Promoter methylation of *FANCN* (PALB2), which represents the partner and localizer of BRCA2 was reported in 2/8 inherited breast tumors, 4/60 sporadic breast tumors, as well as in 4/53 sporadic ovarian tumors [107].

Mutations in the *BRCA1* gene (Breast Cancer 1, early-onset) highly predispose to the development of certain types of cancers such as breast, ovary and prostate cancer. Similar to mutational inactivation of *BRCA1*, promoter methylation of *BRCA1* is also highly associated with sporadic breast cancer, as summarized in a meta-analysis of 40 studies [108]. Initially, *BRCA1* promoter methylation was observed in sporadic cancer samples by South-western analysis [109]. A more extensive work identified a promoter region (nucleotides –567 to +26) that contains 30 CpG dinucleotides. Methylation of this region was observed in a sporadic breast cancer cell line [110]. Methylation of the region upstream of –728 is not associated with *BRCA1* expression. Only methylation of the region downstream of –728 was associated with histone hypoacetylation, chromatin condensation and silencing of the gene in tumor cell lines [111] and in 3 out of 21 breast cancer samples [112]. Up to now methylation of the *BRCA1* promoter was observed in breast cancer [45,101,113–115] and in ovarian cancer [45,114–116] where it was associated with poor patient outcome [117]. Further analysis showed that *BRCA1* promoter methylation also represents a favorable independent prognostic factor in breast cancer patients [118,119].

Besides breast and ovarian cancer, hypermethylation of the *BRCA1* promoter was observed in lung cancer [45,120], gastric cancer [115], NSCL [45,97], uterine cancer [121], and bladder cancer [122].

In addition to hypermethylation, also hypomethylation of genes coding for interstrand crosslink repair was reported. Thus 3/58 primary laryngeal cases showed hypomethylation of *BRCA1* and all tumors showed at the same time hypomethylation in the promoter of the *FANCA* gene [123].

### 5.5. Epigenetic regulation of base excision repair (BER)

Defects in NER, ICL and DDR are associated with inherited chromosomal instability syndromes. This seems not to be the case for BER. However, alterations in BER activity are linked to a variety of neurodegenerative diseases like Alzheimer disease, multiple sclerosis (MS) and Parkinson [124]. They also seem to be linked to aging [124]. The role of BER in cancer formation is less substantiated by experimental evidence. Whereas enhanced levels of 8-OxoG have been found in multiple tumors, no mutations of BER genes have been detected. This could be due to the fact that at least in mice, knockout of key BER proteins is embryonic lethal. However, in several cases an association between polymorphisms in BER genes and cancer incidence has been found. It has also been reported that imbalances in BER gene expression are associated with cancer formation or progression [125].

Concerning epigenetic regulation of BER genes, only limited data are available. They pertain notably the regulation of the DNA glycosylases MBD4, MPG, OGG1, NEIL1 and TDG. The glycosylase MBD4 removes thymine from T:G mismatches, which are the product of deamination of 5-methylcytosine at CpG sites [126]. Within the *MBD4* promoter, a CpG island was identified that is frequently methylated in ovarian and colorectal cancer cell lines displaying a reduced MBD4 expression [127]. In colorectal cancer methylation of the *MBD4* promoter was observed in 24% of the cases, however no correlation between methylation and tumour stage was observed [127]. The proximal *MPG* (N-methylpurine-DNA glycosylase) promoter contains 17 CpG islands close to the transcriptional start site and their methylation correlates with reduced MPG expression. Promoter methylation of *MPG* was observed in glioblastoma cells, and glioblastoma biopsies expressing MPG at low level showed increased methylation of the *MPG* promoter [128]. The *OGG1* promoter was found to be methylated in 5% of thyroid cancer and in some thyroid cancer cell lines [129], the *TDG* promoter, was found to be methylated in several multiple myeloma cell lines that showed decreased TDG expression compared to normal plasma cells [130]. NEIL1, besides being involved in BER, also mediates DNA demethylation, is silenced by promoter methylation in head and neck squamous cell carcinoma [131]. Besides glycosylases also other genes coding for BER genes can be epigenetically silenced. The human *FEN1* promoter contains 2 CpG islands between the nucleotides -458 to +278 bp [132]. A comparison between breast cancer and corresponding normal tissue revealed increased methylation of the second CpG island in the normal tissue, suggesting that the *FEN1* promoter is not hypermethylated, but rather hypomethylated in tumors [132]. Moreover, a reduced expression of APEX1 observed in patient-derived Huntington disease cells was associated with promoter hypermethylation [133]. The *XRCC1* promoter was hypermethylated in gastric cancer [134] the PARP-1 promoter was found to be hypermethylated in cells exposed to the genotoxin benzene [135].

### 5.6. Epigenetic regulation of DNA repair genes involved in DNA double-strand break repair

DNA double strand breaks are repaired by non-homologous end-joining (NHEJ) or homologous recombination (HR). In the process of NHEJ, the protein Ku80 (XRCC5) together with Ku70 (XRCC6) and the

catalytic subunit of the DNA-dependent protein kinase (DNA-PKcs) form a complex that detects DSB. In this complex DNA-PKcs is activated and mediates repair via regulation of additional factors like Artemis, XLF, XRCC4. Analysis of the human *KU80* promoter revealed the presence of two Sp1 sites at +43 and +133 and the presence of a 21 bp palindromic sequence that is located 5' to the distal Sp1 element. These elements, which are required for Ku80 expression, can be silenced by methylation [136]. Methylation of the *ku80* promoter was observed in lung cancer [120]. This has also been observed and supposed to be the molecular cause for silencing of Ku80 in mature neurocytes [137].

During HR, the damaged chromosome enters into physical contact with an undamaged DNA molecule with which it shares sequence homology and which is used as template for repair. HR is initiated by nucleolytic resection of the DSB by the MRE11/Rad50/NBS1 complex. The resulting single-stranded DNA is thereafter covered by RPA protein, which protects against exonucleolytic digestion. Thereafter RAD52 and/or BRCA2 mediate loading of RAD51 onto RPA bound ssDNA and stimulate RAD51-mediated strand invasion. The assembly of the RAD51 nucleoprotein filament is further facilitated by five different paralogues of Rad51 (Rad51B, C and D; and XRCC2 and XRCC3). Similar to *BRCA1*, mutations in the *BRCA2* gene are highly correlated with the development of cancers such as breast, ovary and prostate cancer. In initial experiments, methylation of the *BRCA2* promoter [138] could neither be observed in ovarian cancer cell lines nor in primary sporadic breast cancers [139] and sporadic epithelial ovarian tumors [140]. Recently, however, hypermethylation of the *BRCA2* promoter was detected in breast carcinoma [141,142], ductal carcinoma [143], NSCLC [120] and in laryngeal squamous cell carcinoma [123]. The discrepancy in the findings might be explained by different CpG sites analyzed. Thus it was shown that only the promoter region between the nucleotides -59 and +96, but not the region from +96 to +103, showed methylation in sporadic breast cancer [144]. Besides *BRCA2* promoter methylation, hypermethylation of RAD51C was observed in 0.5% of patients with familial ovarian cancer and early-onset sporadic breast cancer [145].

Werner syndrome ATP-dependent helicase (WRN) represents a member of the RecQ Helicase family, which unwind and separate double-stranded DNA. It further contains a 3' to 5' exonuclease activity that is used for degradation of processed 3' ends and initiation of DNA degradation from a gap in dsDNA. WRN plays an important role in both the repair of double stranded breaks and BER. Methylation of the *WRN* promoter was extensively analyzed in 630 primary tumors corresponding to 11 different tissue types and observed at highest level in colorectal cancer (37.9%), NSCLC (37.5%), and chondrosarcoma (33.3%) followed by gastric cancer (25%), non-Hodgkin lymphoma (23.7%), prostate cancer (20%), breast cancer (17.2%), thyroid tumors (12.5%), osteosarcoma (11.1%), acute lymphoblastic leukemia (9.5%) and acute myeloblastic leukemia (4.8%) [146]. For colorectal cancer it has been shown that *WRN* promoter methylation confers sensitivity to the anticancer drug irinotecan [146]. Additionally, *WRN* promoter has also been shown to be methylated in OSCC [147].

### 5.7. Epigenetic regulation of DNA repair genes associated with the DNA damage response

Following DNA damage, the IP3-like kinases ATM and ATR become activated and phosphorylate the downstream kinases CHK2 and CHK1 and a multitude of other substrates including p53 and several DNA repair proteins (for review see [148]). Mutations in ATM and ATR are responsible for the chromosomal instability syndromes, *ataxia telangiectasia* and *Seckel syndrome*, respectively, which are characterized by high cancer incidence, neurodegeneration and malformations.

Hypermethylation of the *ATM* promoter was first observed in radiosensitive colorectal cells by methylation specific southwestern analysis using two HpaII/MspI sites located within the promoter [149].

Similar to colorectal tumor cell lines, in glioma lines methylation of the *ATM* promoter was associated with increased radiosensitivity [150]. Furthermore hypermethylation of the *ATM* promoter was reported in HNSCC [151], lung [152], recurring adenomas [153] and breast cancer [154]. The latter, however, could not be confirmed by a second study [155].

Hypermethylation of the *CHK2* promoter depends on a CpG rich region between nucleotides -615 and -411 [156]. In this initial report, hypermethylation of *CHK2* was observed in all lung tumor samples analyzed (n = 10) [156]. Hypermethylation of *CHK2* in lung cancer was verified in a second report, showing promoter methylation in 28.1% of NSCLCs (n = 139) [157]. In this study, the methylation frequency was higher in squamous cell carcinoma than in adenocarcinoma (40.0% vs. 19.0), and was also higher in ever-smokers compared to never-smokers (31.7% vs. 17.1%). In human glioma a 10-fold decreased expression of *CHK2* gene was observed, which was partially due to promoter methylation. In this case hypermethylation was shown to inhibit the Sp1 binding to the promoter, which results in attenuation of Sp1 mediated *CHK2* transcription [158].

## 6. Epigenetics in cell and circadian cycle dependent regulation of DNA repair

DNA repair can be also regulated during the cell cycle and the circadian clock. Concerning the cell cycle dependent regulation of DNA repair factors, it has been shown that 58 DNA repair genes were transcriptionally regulated and 30 DNA repair proteins were translationally regulated during cell cycle progression [159]. However, there is no data indicating on 5-methylcytosine dependent epigenetic mechanisms involved.

The circadian clock, which controls the daily rhythmicity of many biochemical processes has been investigated as to regulating DNA repair [160,161]. However, only nucleotide excision repair seems to be controlled by the circadian clock [162,163]. In this case, the circadian transcription factors CLOCK and BMAL1 bind to the *XPA* promoters and activate its transcription. Besides *XPA*, a weak circadian oscillation (1.2 fold) was reported for *MGMT* [164], the DNA glycosylase *MPG* [165] and *OGG1* [166]. This is likely a response on transcription factor level as no evidence is available that CpG methylation is involved. A circadian clock dependency was also reported for the repair of IR-induced DNA damage in mouse splenocytes. In this case circadian oscillation of *BRCA1/2*, *NEIL1*, *RAD51*, *RAD18*, *XRCC2*, *PMS2*, and *XPA*, was observed [167], which was however not linked to CpG methylation in either one of the genes. It has also been shown that the circadian clock can regulate the DNA sensitivity to UV damage and the efficiency of NER by controlling chromatin condensation through histone acetylation [168]. Although the circadian clock regulators *PER1* and *PER2* are controlled by CpG promoter methylation [169,170], the impact on DNA repair still needs to be elucidated.

## 7. Impact of genotoxic exposures on CpG promoter methylation

Whereas the molecular mechanism underlying the CpG methylation of promoters is well known, it is still unclear whether these processes can be regulated by genotoxic stress, thereby contributing to genotoxin-induced carcinogenesis. Theoretically, epigenetic re-programming might occur in cancer cells during therapy with genotoxic drugs, which could lead to more aggressively growing tumors. Experimental evidence for this is provided by several studies performed *in vitro* and *in vivo*.

In chemical-induced rat lung carcinogenesis, CpG promoter hypermethylation of *ERCC1*, *XRCC1*, and *MLH1* increases gradually [171], suggesting that genotoxic stress might contribute to epigenetic silencing of these repair genes. Furthermore, during cadmium-induced malignant transformation, an increase in global DNA methylation was observed, which was associated with overexpression of DNMT1 and

DNMT3a and promoter-methylated silencing of *MSH2*, *ERCC1*, *XRCC1*, and *OGG1* [172]. In hepatocellular carcinoma, *MGMT* promoter methylation was detected in 32 of 83 cases. It was associated with high levels of aflatoxin B1 DNA adducts [173]; the relevance of this finding is unclear. Also during tamoxifen-induced rat liver carcinogenesis a progressive loss of CpG methylation in the regulatory sequences of long interspersed nucleotide elements (LINE-1) was observed. Concomitantly, *RAD51*, *KU70*, and DNA polymerase  $\beta$  (*POLB*) showed increased promoter methylation. These changes in promoter status were associated with decreased protein expression of DNMT1, DNMT3a, DNMT3b [174,175].

*In vitro*, chronic exposure to arsenic and estrogen gave rise to *MLH1* promoter methylation [176] in human prostate epithelial cells, while hypoxia led to hypermethylation of the promoter of *BRCA1* in MCF7 cells [177]. Moreover, UVB-exposure of human lens epithelium B3 cells provoked hypermethylation of the *CSB* promoter and concomitant increase in histone H3K9 deacetylation [92]. It was also reported that low and high LET irradiations induces epigenetic alterations [178]. Thus,  $\gamma$ -radiation leads to global DNA demethylation in human tumor cell lines and the activation of the promoters of *INK4a* and *ATM* [179]. For chemical carcinogens, the hematotoxin benzene was shown to increase the level of *PARP1* promoter methylation and decrease *PARP1* mRNA expression in the lymphoblastoid cell line F32 [135], and following B [a]P exposure multiple promoter hypermethylation events and altered histone modifications were observed in MCF7 cells [180,181].

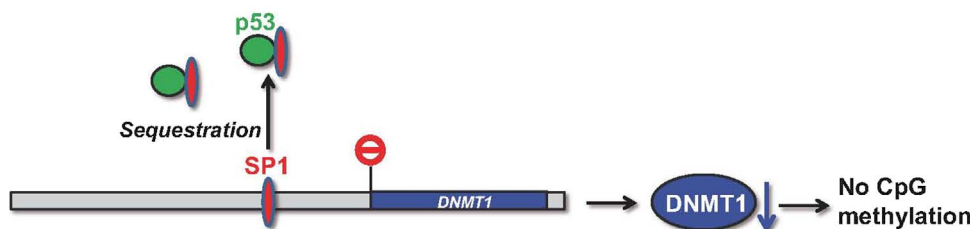
The impact of genotoxic stress on epigenetic alterations was also shown in epidemiological and intervention studies. Enhanced promoter methylation of *MGMT* was observed in peripheral blood lymphocytes of vinyl chloride exposed workers [182] and hypomethylation of the *ERCC2* promoter was observed in arsenic-exposed individuals [183]. Furthermore, workers exposed to diesel engine exhaust exhibited a reduced *MGMT* promoter methylation level in peripheral lymphocytes (although the *MGMT* promoter in lymphocytes is active and considered to be unmethylated) [184]. In twins with discordant smoking habits, smoking-related DNA methylation changes in 22 CpG sites was reported [185], however no alterations in the promoter methylation of *MGMT* and *BRCA1* was observed [186]. Besides carcinogens also vitamin and antioxidant rich diet enhanced the CpG methylation of *MLH1* and *MGMT* in blood samples of human volunteers [187]. Further, supplementation of pregnant pigs with a diet enriched in antioxidants and other nutrients led to reduced oxidative DNA damage and *APE1* promoter methylation in the hippocampus of the newborns [188]. Collectively, the available data suggest that long-term genotoxic exposures have an impact on the methylation status of DNA repair genes.

## 8. Regulation of DNMT1 by p53 following genotoxic stress

How can CpG methylation be regulated by genotoxic stress? A possible mechanism we would like to propose on the basis of available data rests on the regulation of DNMT1 by p53 (Fig. 4). DNMT1 undergoes multiple protein interactions and post-translational modifications, leading to activation, stabilization and recruitment to specific sites in the chromatin [189]. Among its regulators are proteins activated by DNA damage such as p53, GADD45a and PARP-1. A main player in the DNA damage response, p53, has the ability to bind to the promoter of *DNMT1* and thereby acting, contrary to its well-known transcriptional activating function, as a transcriptional repressor [190]. This negative regulation is caused by sequestration of the DNA bound transcription factor Sp1 [191] (as outlined in Fig. 4A). This results in a reduced level of DNMT1 expression and impaired CpG methylation of the genome. If this is a key mechanism, p53 upregulation following genotoxic stress is anticipated to be related to activation of silenced genes. Whether this is the case needs to be shown. Although the impact of genotoxic stress on p53 regulating DNMT1 has been addressed in some *in vitro* studies, the data are conflicting. Thus, IR and etoposide



### A Transcriptional regulation of DNMT1



### B Post-translational regulation of DNMT1

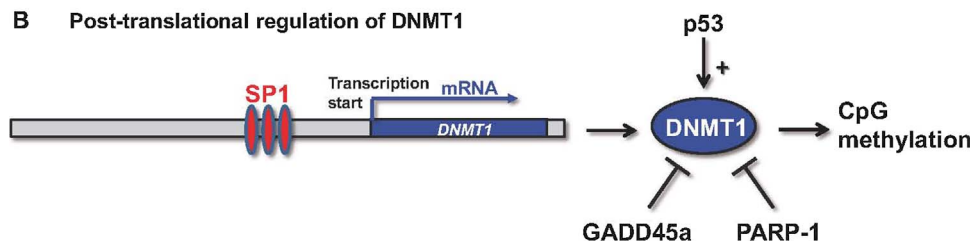


Fig. 4. Mechanism of p53-mediated regulation of DNMT1. A) transcriptional regulation, B) post-translational regulation.

prevented p53 from binding to the promoter of DNMT1 leading to an increase in DNMT1 [190]. Another report shows that MMS, but not MNU, depletes DNMT1 protein, which seems to be mediated through p53 independent degradation of DNMT1 [192]. The situation becomes more complicated as p53 and DNMT1 can directly interact, which stimulates DNMT1 activity and increases the DNMT1-mediated DNA methylation level [193] (Fig. 4B).

There are numerous studies demonstrating an association between p53 status and CpG promoter methylation. Thus, deletion of p53 in the colon cancer cell line HCT116, and knockdown of p53 in TK6 cells, resulted in increased DNMT1 expression [190,194]. Mutations in p53 were associated with DNMT1 overexpression in lung tumors [191]. Overexpression of DNMT1 correlated with the methylation status of promoters of MLH1, MGMT, BRCA1 in pancreatic cancer [195]. Additionally, the methylation frequency of the *MGMT* promoter was higher in p53 mutated lung cancers (62%) than in lung cancer harboring the p53 wild-type (wt) allele (38%) [196]. Similar to lung cancer, an association between *MGMT* promoter hypermethylation and p53 mutation was found in brain cancers (glioblastoma multiforme) [197]. Further support for the role of p53 in the methylation status of *MGMT* promoter was reported in a study where p53 knockdown increased the expression of DNMT1 and histone deacetylase 1 (HDAC1), which resulted in chromatin de-condensation DNMT1 mediated methylation of the *MGMT* promoter, while over-expression of p53 reduced the methylation level of the *MGMT* promoter [196].

A key player in the genotoxic response is PARP-1, which becomes enzymatically activated following binding to DNA repair intermediates, including single-stranded DNA [2]. Inhibition of PARP-1 resulted in aberrant methylation of a CpG island located in the promoter of the *Htf9 gene* [198]. This could result from PARP-1 interaction with DNMT1, blocking its catalytic activity (Fig. 4B) and thereby preventing CpG islands from becoming hypermethylated [199]. A factor involved in activation of PARP-1 and inhibition of DNMT1 is the chromatin insulator, or transcriptional repressor, CTCF [200]. DNMT1 and PARP-1 can interact with CTCF, preventing methylation of CTCF targets [201]. Contrary to the stimulating effect of p53 on DNMT1, the interaction of DNMT1 with GADD45a inhibits its activity [202] (Fig. 4B). The precise mechanism is unknown.

It is important to note that p53 and GADD45a are upregulated presumably by any type of genotoxic stress, p53 on protein [203] and GADD45a on gene level [204]. If both have an impact on the regulation of DNMT1, one might expect that genotoxic stress has a dramatic

impact on the DNA methylation level. This, however, seems not to be the case. There is no evidence that genotoxic stress immediately alters the amount of 5-methylcytosine in the DNA. It also does not cause a change in the promoter methylation level of specific genes. For example, *MGMT* silencing in glioblastoma cells cannot simply be reverted by treatment with alkylating agents or IR, although under these conditions of treatment p53 and GADD45a were induced (unpublished data). It is conceivable, however, that chronic genotoxic exposure has a more profound effect on promoter methylation/demethylation than acute genotoxin treatment.

### 9. Regulation of DNA repair genes by HDACs and SIRT6

Although this review focuses on regulation of DNA repair by CpG promoter methylation, we briefly comment on the role of histone modifications, which can have an influence on the expression of DNA repair genes. Although the impact of histone modifications on the expression of DNA repair genes is difficult to assess, several studies indicate that alterations in histone composition have an impact on repair gene expression. Thus, sodium butyrate, which inhibits class I and class IIA HDACs induces hyperacetylation of histone H4. This led to downregulation of KU70, KU80 and DNA-PKcs mRNA and protein in melanoma cells, causing sensitization to ionizing radiation [205]. Downregulation of these DNA repair proteins was also observed following treatment with the pan-HDAC inhibitor trichostatin A in non-small cell lung cancer cells [206]. In line with this, the pan-HDAC inhibitor SAHA caused downregulation of Ku80 in osteosarcoma and rhabdomyosarcoma cells and sensitized them to ionizing radiation [207]. Besides NHEJ, also HR can be regulated by HDACs. Thus, inhibition of class I HDACs in prostate cancer cells by valproic acid reduced the expression of genes involved in HR such as RAD51, CHK1 and BRCA1. This was caused by reduced recruitment of the transcription factor E2F1 to the promoter of the corresponding genes, which led to increased sensitization to radiation and chemical genotoxins [208]. BRCA1 was also reported to be downregulated by trichostatin A, leading to enhanced radiation sensitivity of carcinoma cells [209]. Decreased expression of RAD51 and reduced HR activity was also observed in malignant melanoma cells upon pharmacologic inhibition of class I HDACs (by MS-275; entinostat) and knockdown of HDAC2, which resulted in inefficient repair of DSBs and sensitization to temozolomide [210].

## 10. Conclusions

DNA repair is essential for maintaining genomic stability and protecting cells against endogenous and exogenous DNA damaging insults. It is also required for specific cellular processes such as immunoglobulin diversification. DNA repair is tightly tissue-specific regulated and frequently deregulated in cancer cells. Regulation of DNA repair occurs on post-translational, post-transcriptional (via miRNA) and transcriptional level. The latter involves transcription factors like p53, AP-1 and NF- $\kappa$ B, which regulate DNA repair during the immediate stress response. It is also regulated epigenetically by 5-methylcytosine in CpG islands in the promoter and by histone modifications. The repair gene most extensively regulated by CpG promoter methylation is MGMT. This corresponds in the hypermethylated status with gene silencing, lack or low level of expression of MGMT protein, drug sensitivity and a better outcome following alkylation based therapy. While MGMT is promoter methylated in up to 45% of brain cancers and other tumor groups, other repair genes are less frequently silenced by promoter methylation. The reason for this is unclear. CpG methylation requires *de novo* methyltransferases (DNMT) while demethylation is dependent on TET and base excision repair enzymes. Thus, on the one hand DNA repair is required for regulating the epigenetic switch and, on the other, it is itself subject of epigenetic regulation. While acute DNA damage does not immediately change the CpG methylation level, there are examples that chronic exposure has an impact on epigenetic reprogramming. Although it is well established that epigenetic silencing of repair genes like MGMT has a profound effect on cancer therapy with monofunctional alkylating agents, its impact on therapy with other anticancer drugs remains to be elucidated. It also remains to be seen to what extent epigenetic silencing of repair genes determines tissue specific differences in repair capacity and contributes to ageing and genotoxin triggered diseases.

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