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18 DNA Repair and Epigenetics in Cancer

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18.1 DNA REPAIR IN CANCER

Cells are under constant genotoxic pressure from both endogenous and exogenous sources. It has been estimated that every day a single human cell has to endure tens of thousands of DNA lesions (Jackson and Bartek 2009). This damage needs to be repaired to avoid detrimental mutations, blockage of replication and transcription, and chromosomal breakage. DNA repair is the collection of the multiple and diverse ways through which living cells identify alterations in the chemistry of their DNA molecules and correct the damage to restore the integrity of their genome. In cancer, DNA repair serves as a significant barrier that can prevent preneoplastic cells from progressing through malignant transformation. The importance of DNA repair in preventing cancer was first demonstrated in the study of patients with xeroderma pigmentosum (XP), a rare autosomal recessive genetic disorder characterized by extreme sensitivity to ultraviolet (UV) rays caused by a deficiency in the ability to repair damage caused by sunlight (Cleaver 1968, Setlow et al. 1969). Individuals with XP exhibit skin malignancies and cancer at a young age. Further support for the critical role of DNA repair in preventing cancer in humans came from the discovery of other DNA repair mechanisms, summarized in this chapter (Figure 18.1). The mechanism through which DNA is repaired depends on the type and extent of the DNA damage. In mammalian cells, there are six major DNA repair pathways with unique—but sometimes overlapping—functions, to mend the damage caused by exogenous DNA-damaging agents (including chemotherapy and radiotherapy) and damage caused by normal endogenous cellular processes (Kelley and Fishel 2008).

18.1.1 Direct Reversal (DR)

In humans, there is only one type of DNA damage that can be repaired by direct chemical reversal. This mechanism can only repair one type of lesion and does not involve breakage of the phosphodiester backbone; thus, not requiring a template for the repair. The DR pathway removes alkyl groups (CH_3-) at the O^6 position of guanine by direct transfer to O^6-methylguanine-DNA methyltransferase (MGMT) (Tano et al. 1990, Natarajan et al. 1992). MGMT transfers the methyl group to a cysteine residue in the protein. In this process, each MGMT molecule can only be used once. Impairment of the DR pathway would allow the O^6-methylguanine to pair with thymine instead of cytosine, leading to G to A mutations (Kaina et al. 2007). When MGMT is unsuccessful in removing O^6-methylguanine during DR, the mismatch repair (MMR) pathway can recognize and fix the resulting O^6-methylguanine mispairs (Luo et al. 2010). Interestingly, glioma patients with *MGMT* gene inactivation, which would render the tumors incapable of repairing O^6-methylguanine, have better survival rates than patients with active *MGMT* following treatment with alkylating agents such as carmustine and temozolomide (Esteller et al. 2000, Hegi et al. 2005). As one would predict, lack of MMR has also been shown to render tumors resistant to alkylating agents, even in the absence of MGMT (Liu, Markowitz, and Gerson 1996).

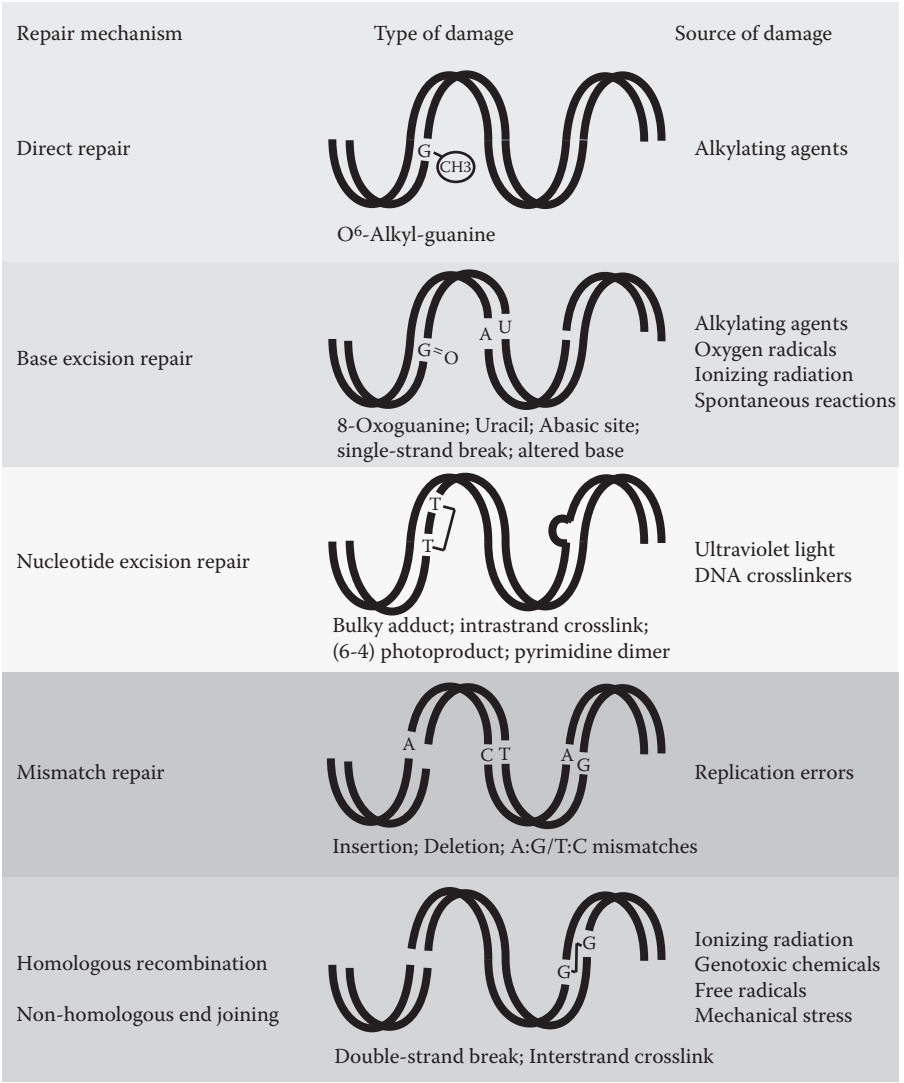

FIGURE 18.1 DNA repair pathways in humans. DNA repair pathways and their corresponding type of DNA damage and sources of endogenous and exogenous agents are summarized in this figure.

18.1.2 Base Excision Repair (BER)

BER is one of three excision repair pathways that happen to repair single stranded DNA damage. BER removes small, non-bulky lesions (do not distort the DNA helix) produced by alkylation, oxidation or deamination of bases. In this DNA repair mechanism, a DNA glycosylase-type enzyme removes a single damaged DNA base,

forming an apurinic/apyrimidinic site (AP site). Additional steps including DNA backbone incision, gap filling, and ligation then repair the resulting AP site. Thus, a characteristic of BER is the diversity of the DNA glycosylases, which recognize specific substrates. Either the short-patch (single nucleotide replacement) or long-patch (two to eight nucleotides are synthesized) BER pathway can process the resulting single-strand break that results after cleavage by AP endonucleases. Short-patch BER repairs most AP sites, while oxidized and reduced AP sites are preferentially repaired through the long-patch pathway. BER is important to removing damaged bases that could lead to mutations by base mispairing or lead to breaks in DNA during replication.

In human cancer, C to T transition mutations at CpG dinucleotide sites are the most common kind of genetic alteration. In part, these mutations arise from the spontaneous deamination of methylated cytosines (5-methylcytosine) (Pfeifer 2006). Methyl-CpG binding domain protein 4 (MBD4) and thymine DNA glycosylase (TDG) are two BER glycosylases responsible for binding and removing mismatched thymine from DNA (Hendrich et al. 1999, Yoon et al. 2003). Mutations in MBD4, but not TDG, have been observed with cancers with genomic instability (Bader et al. 1999).

Another BER enzyme which, when mutated, may be involved in carcinogenesis is OGG1 (Chevillard et al. 1998, Shinmura and Yokota 2001). OGG1 is the glycosylase responsible for the excision of 8-oxoguanine, a mutagenic base byproduct that occurs as a result of exposure to reactive oxygen (Arai et al. 1997). Unrepaired 8-oxoguanines lead to G to T or G to C transversions.

18.1.3 Nucleotide Excision Repair (NER)

NER is another excision repair pathway involved in the repair of single stranded DNA damage. In NER, large adduct and bulky DNA lesions that cause a significant distortion of the DNA double helix are excised within a string of nucleotides and replaced with DNA as directed by the undamaged template strands. Thus, NER is the DNA repair mechanism used only when one of the two DNA strands is disturbed. This type of damage usually occurs as a result of cross-linking agents (e.g. UV radiation) and base-damaging carcinogens (Luo et al. 2010).

NER is a multi-step repair process that involves more than 30 proteins, listed in Table 18.1. There are two NER sub-pathways: global genomic repair (GGR) and transcription coupled repair (TCR). GGR acts throughout the genome, regardless of whether the specific sequence is the transcribed or non-transcribed strand of a gene (Sugasawa et al. 2001, Riedl, Hanaoka, and Egly 2003). As the name indicates, the TCR repair machinery removes lesions only from the transcribed strand of active genes, removing distorting lesions that block transcriptional elongation by RNA polymerases (Fousteri and Mullenders 2008, Hanawalt and Spivak 2008). The protein complexes that recognize the DNA damage site and initiates DNA repair determine the NER sub-pathway selection (Luo et al. 2010).

TABLE 18.1
NER Associated Genes

Human Gene	Protein	Subpathway	Function in NER
CCNH	Cyclin H	Both	CDK Activator Kinase (CAK) subunit
CDK7	Cyclin Dependent Kinase (CDK)7	Both	CAK subunit
CETN2	Centrin-2	GGR	Damage recognition; forms complex with XPC
DDB1	DDB1	GGR	Damage recognition; forms complex with DDB2
DDB2	DDB2	GGR	Damage recognition; recruits XPC
ERCC1	ERCC1	Both	Involved in incision on 3' side of damage; forms complex with XPF
ERCC2	XPD	Both	ATPase and helicase activity; transcription factor II H (TFIIH) subunit
ERCC3	XPB	Both	ATPase and helicase activity; transcription factor II H (TFIIH) subunit
ERCC4	XPF	Both	Involved in incision on 3' side of damage; structure specific endonuclease
ERCC5	XPG	Both	Involved in incision on 5' side of damage; stabilizes TFIIH; structure specific endonuclease
ERCC6	CSB	TCR	Transcription elongation factor; involved in transcription coupling and chromatin remodeling
ERCC8	CSA	TCR	Ubiquitin ligase complex; interacts with CSB and p44 of TFIIH
LIG1	DNA Ligase I	Both	Final ligation
MNAT1	MNAT1	Both	Stabilizes CAK complex
MMS19	MMS19	Both	Interacts with XPD and XPB subunits of TFIIH helicases
RAD23A	RAD23A	GGR	Damage recognition; forms complex with XPC
RAD23B	RAD23B	GGR	Damage recognition, forms complex with XPC
RPA1	RPA1	Both	Subunit of RFA complex
RPA2	RPA2	Both	Subunit of RFA complex
TFIIH	Transcription factor II H	Both	Involved in incision, forms complex around lesion
XAB2	XAB2	TCR	Damage recognition; interacts with XPA, CSA, and CSB
XPA	XPA	Both	Damage recognition
XPC	XPC	GGR	Damage recognition

The xeroderma pigmentosum group C protein, encoded by the XPC gene is a subunit of these damage recognition complexes and is essential for GGR (Friedberg 2001, Riedl, Hanaoka, and Egly 2003). For the TCR pathway, recognition of the DNA damage-blocked RNA polymerase by transcription-repair coupling factors is important. After damage recognition, both NER subclasses have the same or similar subsequent steps involved in nucleotide excision and gap filling by DNA polymerases.

18.1.4 Mismatch Repair (MMR)

The last excision repair pathway involved in the repair of single stranded DNA damage is MMR. During DNA replication mistakes can occur that escape the proofreading activity of DNA polymerase as it copies the two strands. The MMR pathway is responsible for recognizing and repairing single-base insertions, deletions, and mismatches that arise during normal DNA replication process (Luo et al. 2010, Fleck and Nielsen 2004). These errors that escape the proofreading activity of DNA polymerases happen with a frequency of about 1 in 10^9–10^{10} base pairs per cell division (Iyer et al. 2006). Furthermore, exposure to exogenous agents or endogenous reactive species may cause base modifications that lead to nucleotide mispairing (Li 2008). Loss of MMR affects genome stability (including microsatellite instability), which causes cancer predisposition (Jiricny 2006). In this pathway, PMS2, MLH1, LSH6, and MSH2 are proteins that recruit EXO1 to excise the segment of mutant DNA strand. Then DNA polymerases replace the missing section of the strand with a new section and the damage is repaired. The vast majority of hereditary non-polyposis colorectal cancers (HNPCC) are attributed to mutations in the *MSH2* and *MLH1* genes (Bronner et al. 1994).

18.1.5 Homologous Recombination (HR)

HR is one of two mechanisms through which DNA double-strand breaks can be repaired. DNA damage that has not been repaired before replication can cause DNA polymerase blockage, resulting in DNA double-strand breaks. HR is the repair pathway used to fix double-strand breaks detected during the S/G2 phases of the cell cycle, when a homologous template via the sister chromatid is available. Since HR requires a long homologous sequence to guide the repair, it is highly accurate in its repair (Fleck and Nielsen 2004). The DNA checkpoint responses are responsible for the regulation of double-strand break ends processing, which will determine which DNA double-strand break repair mechanism will perform the repair. This is a crucial stage in the recombination process (Lazzaro et al. 2009).

Two of the most studied genes and proteins that are involved in this repair pathway are BRCA1 and BRCA2. These tumor suppressor proteins form a complex along with RAD51 to repair DNA double-strand breaks (Duncan, Reeves, and Cooke 1998, Yoshida and Miki 2004). Cells missing BRCA1 and BRCA2 have a decreased

rate of HR. Mutations in the *BRCA1* and *BRCA2* genes have been associated with considerably increased risk for breast and ovarian cancer (Miki et al. 1994, Wooster et al. 1994).

18.1.6 Non-Homologous End Joining (NHEJ)

NHEJ is the other pathway that repairs double-strand breaks in DNA. Unlike HR, NHEJ has the potential to relegate any type of DNA ends, without the need for a homologous template. Since NHEJ does not require an identical copy of DNA as a template, it is not restricted to a certain phase of the cell cycle, and it is prone to imprecise repair leading to loss or addition of bases in the ligation process (Fleck and Nielsen 2004). Inactivation of CDK1 increases NHEJ events in the G2 phase of the cell cycle (Lazzaro et al. 2009). DNA strands that are not repaired completely by NHEJ are subject to repair by HR (Essers et al. 2000).

The initial step in NHEJ is the recognition and binding of the Ku heterodimer at the DNA double-strand break (Mari et al. 2006). The Ku heterodimer is composed of Ku70 and Ku80, encoded by the *XRCC6* and *XRCC5* genes, respectively. Once the Ku heterodimer is bound the DNA double-strand break ends, it serves as a scaffold to recruit the other NHEJ factors to the damage site. No spontaneous Ku mutations have been found in humans, suggesting that both Ku70 and Ku80 are likely required for viability.

18.2 EPIGENETICS IN CANCER

It was long thought that tumorigenesis was mostly driven by genetic mutations and genomic instability. With the advent of whole-genome sequencing, cancers with a low rate of mutations have been identified and epigenetics has gained an ever-increasing role in the process of tumor progression (Zhang et al. 2012, Feinberg, Koldobskiy, and Gondor 2016). Epigenetics is defined as the inheritable changes in gene expression with no alterations in DNA sequences. During the past few years several studies showed the connection between disruptions of the epigenome, defined as the combination of changes in gene expression, and tumor progression. In the eukaryotic nucleus, DNA is compacted into a chromatin structure with the nucleosome as the basic unit, in which 147 bases of DNA surround each histone octamer. The histone octamer includes two elements of the core histone (H3, H4, H2A, and H2B) (Luger et al. 1997). Unlike the other histones H1, the “linker “histone, is not a component of the nucleosome. It interacts at the DNA entrance and exit site of the nucleosome and the linker DNA that connects adjacent nucleosome. There are three main epigenetic modifications that regulate chromatin structure and gene expression: DNA methylation, histone covalent modification and microRNAs (miRNAs) (Figure 18.2). All together, they constitute the “epigenetic code,” that is capable of modulating the expression of the different cell types. Disruption of epigenetic processes can lead to altered gene function and malignant cellular transformation (Sharma, Kelly, and Jones 2010).

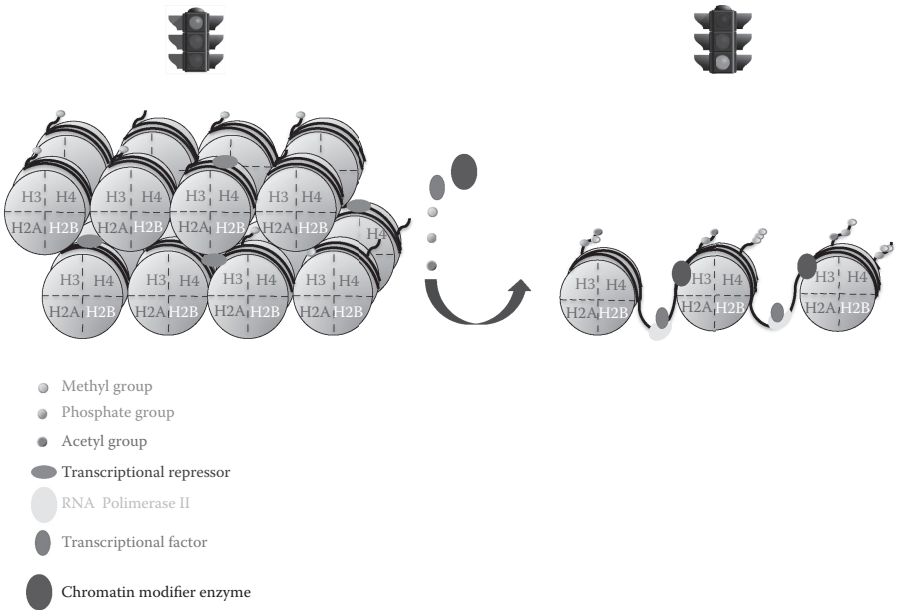

FIGURE 18.2 Euchromatin and heterochromatin landscape. Schematic representation of the nucleosome octamers. Condensed chromatin is not accessible to transcription factors (repressed chromatin, left). Epigenetics modifiers can render chromatin accessible to transcription factors and RNA polymerase II (active chromatin, right).

18.2.1 DNA Methylation

DNA methylation is a covalent modification of DNA that has been described in bacteria, plants, and mammals. It can occur following DNA replication, in order to re-establish the preexisting DNA methylation pattern or *de novo*, and in both situations acts to repress gene transcription (Chen et al. 2014). In eukaryotic cells, the 5' methyl group is added to the cytosine base, and this modification is most frequently found in the context of CpG dinucleotides. S-adenosyl-methionine is the methyl donor in a reaction catalyzed by the DNA methyltransferase (DNMT) family, including DNMT1, DNMT3A and DNMT3B. DNMT1 is responsible for the methylation of hemi-methylated DNA and thus DNA methylation maintenance, whereas DNMT3A and DNMT3B are involved in *de novo* DNA methylation, but they can also participate in methylation maintenance (Castillo-Aguilera et al. 2017). It can be speculated that DNA methylation is capable of preventing gene transcription either by blocking the combination of a transcription factor and its binding sites, or through the recruitment of methylated binding domain proteins that mediate inhibition of gene expression.

In some areas of the genome, CpG sites are concentrated in short CpG-rich DNA fragments or DNA fragments in the long repeat so-called 'CpG islands'. CpG island-containing gene promoters are usually un-methylated in normal cells to maintain euchromatic structure, which is the transcriptional active conformation allowing gene expression (Chen et al. 2014).

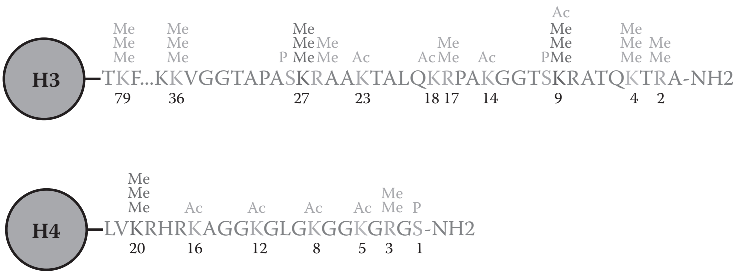

FIGURE 18.3 Predominant post-translational modifications of histones H3 and H4. Partial view of histones H3 and H4 N-terminal tail with the main post-translational modifications known to lead gene transcriptional activation or repression. The corresponding residue number is shown under each modified amino acid. Marked in green are the amino acids and post-translational modifications that lead to transcriptional activation. In red are the amino acid residues and post-translational modifications that lead to transcriptional repression. In purple is the amino acid that can be modified to both active and repressive transcription. Me = methyl group, P = phosphate group, Ac = acetyl group.

18.2.2 Histone Modifications

The histone octamer, the basic element of the nucleosome core particle, consists of two copies of each core histone proteins (H2A, H2B, H3, and H4). The N-terminals of histones protrude out of the nucleosome core, and amino acids of N-terminals easily undergo a series of covalent modifications, such as methylation, acetylation, phosphorylation, ubiquitination and sumoylation (Figure 18.3) (Tessarz and Kouzarides 2014, Cheung, Allis, and Sassone-Corsi 2000). These post-translational modifications can regulate important processes such as gene transcription, X-chromosome inactivation, mitosis, heterochromatin formation, DNA repair, and replication (Kouzarides 2007). Regarding gene transcription, histone H3 lysine 9 acetylation (H3K9ac), H3 serine 10 phosphorylation (H3S10ph), and H3 lysine 4 tri-methylation (H3K4me3) are reported to be associated with transcriptional activation. Conversely, H3K27me3 and hypoacetylation of H3 and H4 are correlated with transcriptional repression. Importantly, a large body of evidence supports a scenario in which combinatorial modifications correspond to specific functional chromatin states. Individual post-translational modifications can favor or inhibit consequent modifications on nearby residues of the same tail (Fischle, Wang, and Allis 2003, Latham and Dent 2007). For example phosphorylation of Ser-10 on H3, is a positive signal for subsequent acetylation at K14 on the same tail (Lo et al. 2000, Cheung, Allis, and Sassone-Corsi 2000) whereas histone deacetylation and methylation of H3-K9 lysine represses transcription (Fuks 2005).

18.2.3 microRNAs

miRNA encode small noncoding RNA molecules (19–25 nucleotides in length) that are complementary to the 3’ untranslated regions of target mRNAs. This results in

gene silencing through translational repression or target mRNA degradation (Bartel 2009). Studies on miRNAs have demonstrated how they regulate gene expression also at transcriptional level, and not only at post-transcriptional level as described above. Promoter-associated RNA (paRNA) can also regulate transcription of genes by targeting the promoter (Costa 2010). PaRNA can also modify the recruitment of the epigenetic machinery to enhance or silencing transcription of mRNA (Yan and Ma 2012).

18.2.4 The Epigenetic Classification System for Cancer Genes

The old classification of cancer genes into dominant oncogenes (*MYC, KRAS, PIK3CA, ABL1, BRAF*) and recessive tumor suppressor genes (*RB1, TP53, WT1, NF2, VHL, APC, CDKN2A*), has been replaced by the more functional epigenetics classification of the cancer genes, which includes the epigenetic modifiers, the mediators and the modulators (Feinberg, Koldobskiy, and Gondor 2016). Epigenetic modifiers are gene products capable of directly modifying the epigenome through DNA methylation, post-translational modification of chromatin, or the alteration of the structure of the chromatin. The epigenetic mediators are often the targets of epigenetic modification, although they are rarely mutated themselves; importantly, they appear to be responsible for the emergence of cancer stem cells (Feinberg, Koldobskiy, and Gondor 2016). Finally, the epigenetics modulators are defined as genes lying upstream of the modifiers and mediators in signaling and metabolic pathways, and serving as the mechanism by which environmental agents, injury, inflammation, and other forms of stress push tissues towards a neoplastic propensity and/or increase the likelihood that cancer will arise when a key mutation occurs by chance.

18.2.5 Epigenetic Modifier Mutations and Cancer

Epigenetic modifier mutations are a common occurrence in a wide range of cancers (Table 18.2). These occur in components at every level of the epigenetic machinery including DNA methylation and histone modification.

18.2.5.1 DNA Methylation

Hematological malignances are highly related to mutations in the DNA methylation machinery. These events clearly underline how epigenetics and genetics can cooperate in cancer initiation and progression. DNA methyltransferase3α (DNMT3A) mutations have been described in human acute myeloid leukemia, acute monocytic leukemia and T-cell lymphoma (Ley et al. 2010, Yan et al. 2011, Couronne, Bastard, and Bernard 2012). Moreover, *DNMT3A* mutations are considered a marker of poor prognosis both in acute myeloid leukemia and T-cell acute lymphoblastic leukemia (Grossmann et al. 2013, Ribeiro et al. 2012). *DNMT1* mutations have been described in colon cancer (Kanai et al. 2003). Mutations in *DNMT3B* have been associated with a rare autosomal recessive immunoglobulin deficiency, sometimes combined with defective cellular immunity called immunodeficiency-centromeric instability-facial anomalies (ICF) syndrome (Wijmenga et al. 2000). In addition, a single nucleotide polymorphism (SNPs) that involves a C to T transition on the promoter of this gene

TABLE 18.2
Epigenetic Modifier Mutations in Cancer

Gene	Function	Cancer
DNMT3A	DNA methylation (*de novo*)	Acute myeloid leukemia (Ley et al. 2010); T-cell lymphoma (Couronne, Bastard, and Bernard 2012)
DNMT1	DNA methylation	Colon cancer (Kanai et al. 2003)
DNMT3B	DNA methylation (*de novo*)	Lung adenocarcinoma (Shen et al. 2002); breast cancer (Roll et al. 2008)
Tet2	DNA demethylation	Myelodysplastic syndrome; myeloproliferative neoplasms; acute myeloid leukemia (Gaidzik et al. 2012)
IDH1/2	DNA demethylation	Glioma (Turcan et al. 2012); acute myeloid leukemia (Figueroa et al. 2010)
EP300	Histone acetylation	Acute myeloid leukemia (Wang, Gural et al. 2011)
HDAC1	Histone deacetylase	Prostate cancer (Halkidou et al. 2004); gastric cancer (Choi et al. 2001)
HDAC2	Histone deacetylase	Colorectal cancer (Ozdag et al. 2006)
HDAC4	Histone deacetylase	Breast cancer (Sjoblom et al. 2006)
HDAC7A	Histone deacetylase	Colorectal tumors (Ozdag et al. 2006)
KMT2A	Histone methyltransferase	Acute myeloid leukemia (Thirman et al. 1993)
KMT2B	Histone methyltransferase	Endometrial; large intestine; lung; glioma; liver carcinoma (Rao and Dou 2015)
KMT2C	Histone methyltransferase	Endometrial; large intestine; lung; bladder carcinoma (Rao and Dou 2015)
KMT2D	Histone methyltransferase	Acute myeloid leukemia; lung large intestine carcinoma; bladder carcinoma (Rao and Dou 2015)
EZH2	Histone methyltransferase	Non-Hodgkin lymphoma; solid tumors; T-cell leukemia (Feinberg, Koldobskiy, and Gondor 2016)
NSD1	Histone methyltransferase	Acute myeloid leukemia (Varier and Timmers 2011)
SMYD3	Histone methyltransferase	Colon; breast; hepatocellular carcinoma (Varier and Timmers 2011)
G9A	Histone methyltransferase	Hepatocellular carcinoma (Varier and Timmers 2011)
PRMT1	Arginine methyltransfrase	Breast cancer (Gao et al. 2016)
PRMT5	Arginine methyltransfrase	Hematologic and solid tumors (Tarighat et al. 2016)
LSD1	Histone demethylase	Bladder; colorectal cancer (Rotili and Mai 2011)
KDM6A	Histone demethylase	Myeloma; renal cell carcinoma (Rotili and Mai 2011)
BRCA1	Ubiquitin ligase	Breast; ovarian cancer (Zhu et al. 2011)
USP22	Ubiquitin hydrolase	Breast cancer (Zhang et al. 2011)

has been associated with increased *DNMT3B* promoter activity and increased risk of lung cancer, while *DNMT3B* overexpression can lead to hypermethylation and silencing of key genes in human breast cancer cell lines (Shen et al. 2002, Roll et al. 2008). DNA methylation can be reversed by erasers, which are part of the epigenetic machinery. Among these we can find TET (Ten-eleven-translocation) and AID (Activation-induced cytidine deaminase) demethylases (De Carvalho et al. 2012, Ko et al. 2010, Wu and Zhang 2010). Active DNA demethylation is currently

thought of as being a stepwise process. First 5-methylcytosine (5mC) is converted into 5-hydroxymethylcytosine (5hmC) by the TET family of enzymes. Subsequently 5hmC can be deaminated by the AID/APOBEC family members to form 5-hydroxymethyluracil (5hmU). The DNA excision repair system can finally replace the cytosine without the methyl group (Bhutani, Burns, and Blau 2011). Three TET family members (TET1, TET2, and TET3) have been reported so far and each protein seems to have a distinct function in different cellular contexts (Cimmino et al. 2011). Mutations in TET2 including frame shift, nonsense and missense mutations, have been found in various myeloid neoplasms and gliomas (Gaidzik et al. 2012). Cytosolic isocitrate dehydrogenase 1/2 (*IDH*) mutants display global DNA hypermethylation along with the accumulation of 2-hydroxyglutarate, and they are also capable of impairing TET2 function. *IDH1/2* mutations were mutually exclusive with mutations in the α-ketoglutarate-dependent enzyme *TET2*, while *TET2* loss-of function mutations (a type of mutation in which the altered gene product lacks the molecular function of the wild-type gene mutations) are associated with similar epigenetic defects as *IDH1/2* mutants (Figueroa et al. 2010). *IDH1/2* mutations are described in different kind of gliomas and leukemias (Turcan et al. 2012). These mutants show impaired hematopoietic differentiation (Figueroa et al. 2010), and impaired cell differentiation (Lu et al. 2012).

18.2.5.2 Histone Acetylation and Deacetylation

The acetylation of lysine on histone is generally associated with active gene transcription. Acetyltransferase (HATs) can be grouped into three main categories according to their sequence similarity: Gcn5-related–N-acetyltransferase (GNAT), MYST (acronym for the founding members MOZ, Ybf2, Sas2, TIP60), and orphan (p300/CBP and nuclear receptors) (Yang 2004). Several mutations such as amplifications, point mutations or translocations of HATs have been described. Several publications connected acetyltransferase mutation to different types of cancer. Mutations, translocations or deletions of these genes are observed in colon, uterine, lung tumors, and leukemia (Esteller 2007). HATs can also modulate the activity of fusion proteins. It has been previously described how AML1-ETO, the most frequent fusion protein in acute myeloid leukemia needs p300-mediated site-specific acetylation to drive leukemogenesis (Wang, Gural, et al. 2011).

Histone deacetylases (HDAC) are erasers that can remove acetyl. There are five classes of histone deacetylases:

-class I: HDAC1, HDAC2, HDAC3, and HDAC8
-class IIa: HDAC4, HDAC5, HDAC7, HDAC9
-class IIb: HDAC6 and HDAC10
-class III: Sirtuins (SIRT1-7)
-class IV: HDAC11

Somatic changes in HDAC genes are implicated in cancer progression. *HDAC1, HDAC5* and *SIRT1* are downregulated in some renal, bladder, and colorectal tumors (Ozdag et al. 2006). *HDAC1* is overexpressed in prostate and gastric cancer (Halkidou et al. 2004, Choi et al. 2001). *HDAC2* mutations correlate with colorectal cancer (Ozdag et al. 2006), gastric (Ropero et al. 2006), and endometrial primary

tumors (Ropero et al. 2006). The loss of HDAC2 protein expression renders those cells more resistant to the usual anti-proliferative and pro-apoptotic effect of histone deacetylase inhibitors. *HDAC4* mutations have been identified in breast cancer (Sjoblom et al. 2006). *HDAC5* is overexpressed in some colorectal tumors (Ozdag et al. 2006). And, higher expression of *HDAC7A* was observed in most colorectal tumors (Ozdag et al. 2006). Understanding the role of epigenetics modifiers in cancer can open new avenues for medical treatment through the identification of new drugs that specifically target these factors.

18.2.5.3 Histone Methylation

Methylation of arginine and lysine residues on histone protein tails can regulate chromatin structure and gene expression. One well-known example for alterations in histone methylation is mixed lineage leukemia (MLL). *MLL1* (also known as *KMT2A*) is the human homolog of the *trithorax* (*trx*) in Drosophila. The trithorax group of proteins typically function in large complexes formed with other proteins and are most commonly associated with gene activation. MLL regulates H3K4me3, an active mark for transcription. Translocations of *MLL1* with multiple different partners can originate fusion proteins that have abnormal patterns of H3K4me3 and/or abnormal patterns of histone modifier recruitment resulting in tumorigenesis. Rearrangement of the *MLL1* gene has been described in acute lymphoblastic and acute myeloid leukemia (Thirman et al. 1993). Mutations in *MLL1* have also been described in a large spectrum of solid tumors (colon, lung, bladder, endometrial, and breast cancers) (Rao and Dou 2015). Mutations in the coding region of *MLL2* (also known as *KMT2B*), another member of the MLL family of methylases, occur in endometrial, large intestine, lung, glioma, and liver carcinomas (Rao and Dou 2015). To date, hundreds of *MLL3* (known as *KMT2C*) and *MLL4* (known as *KMT2D*) mutations have been identified, making them among the most frequently mutated genes in human cancer. *MLL3* mutations are prevalent in lung, large intestine, breast, endometrial, and bladder carcinomas. All these together account for 60% of the total *KMT2C* mutations identified (Rao and Dou 2015). Nonsense, missense and frameshift mutation of *MLL4* (known as *KMT2D*) have been related to acute myeloid leukemia, lung, large intestine, endometrial carcinomas, and medulloblastoma (Rao and Dou 2015). *EZH2* (enhancer of zeste homologue 2) is a member the Polycomb repressive complex 2 (PRC2). EZH2 regulates H3K27me3 and there are two different classes of mutations that affect its function. Gain-of-function hotspot mutations (a type of mutation in which the altered gene product possesses a new molecular function or a new pattern of gene expression) and amplification have been reported in non-Hodgkin lymphomas and solid tumors. These events suggest how these tumors require an increased level of H3K27 tri-methylation (Feinberg, Koldobskiy, and Gondor 2016). On the contrary, loss-of-function mutations (a type of mutation in which the altered gene product lacks the molecular function of the wild-type gene) of *EZH2* have been described in myeloid malignancies, head and neck squamous carcinomas, and T-cell leukemia (Feinberg, Koldobskiy, and Gondor 2016). Other lysine histone methyl-transferases (HMTs) are aberrantly expressed in several cancers. NSD1 (Nuclear Receptor Binding SET Domain Protein 1) methylates Lys-36 of histone H3 and Lys-20 of histone H4 (*in vitro*). This transcriptional

intermediary factor is capable of both negatively or positively influencing transcription, depending on the cellular context. NSD1 translocations have been described in acute myeloid leukemia (Varier and Timmers 2011). SMYD3 (SET and MYND Domain Containing 3) specifically methylates Lys-4 of histone H3, inducing di- and tri-methylation, but not mono-methylation. It also methylates Lys-5 of histone H4. SMYD3 overexpression has been described in colon, breast, and hepatocellular carcinoma (Varier and Timmers 2011). G9a regulates mono- and di-methylation of Lys-9 of histone H3 (H3K9me1 and H3K9me2, respectively) in euchromatin. H3K9me represents a specific tag for epigenetic transcriptional repression. G9a overexpression has been detected in hepatocellular carcinoma (Varier and Timmers 2011). Evidence for the role of arginine HMTs in tumorigenesis has not been as well established as that of lysine HMTs. PRMT1 (Protein Arginine Methyltransferase 1) constitutes the main enzyme that mediates mono-methylation and asymmetric di-methylation of histone H4 Arg-3 (H4R3me1 and H4R3me2a, respectively), a specific tag for epigenetic transcriptional activation. Upregulation of PRMT1 expression has been described in breast cancer (Gao et al. 2016). PRMT5 (Protein Arginine Methyltransferase 5) mediates the formation of omega-N mono-methyl-arginine (MMA) and symmetrical di-methylarginine (sDMA). Overexpression of PRMT5 has been reported in hematologic and solid malignancies (mantle cell lymphoma, lung and bladder cancer, gastric cancer, germ cell tumors) (Tarighat et al. 2016). Histone demethylases (KDMs) are erasers responsible for removing histone methylation. Aberrant expression of LSD1 (Lysine-specific histone demethylase 1), that demethylates both Lys-4 (H3K4me) and Lys-9 (H3K9me) of histone H3, has been shown in many types of cancers (bladder, small cell lung, and colorectal cancer) (Rotili and Mai 2011). Downregulation or inactivation of KDM6A/UTX (lysine-specific demethylase 6A), specific for demethylation of H3K27me3/me2, have been reported in various type of cancers such multiple myeloma, esophageal squamous cell carcinomas, and renal cell carcinomas (Rotili and Mai 2011). Another large class of histone demethylases is composed of the Jumonji family of Lysine demethylases. These enzymes can demethylate all three lysine methylation states (tri-, di-, and mono-methylation) at H3K4, H3K9, H3K27, and H3K36, as well as H1K26. Six different subfamilies (JMJD1s, JMJD2s, JARID1s, UTX/Y-JMJD3, PHFs, and FBXLs) of JmjC histone demethylases have been identified, which have different histone sequence and methylation state selectivity. Misregulation of JmjC KDMs has significantly been implicated in cancer initiation and progression (Rotili and Mai 2011).

18.2.5.4 Histone Ubiquitination

Histone H2A was the first protein identified to be modified by ubiquitination (Goldknopf et al. 1975). H2A and H2B are two of the most abundant ubiquitinated proteins present in the nucleus (5–15% for H2A and 1–2% for H2B) (Goldknopf et al. 1975, West and Bonner 1980, Robzyk, Recht, and Osley 2000). The most frequent forms of histone ubiquitination are mono-ubiquitination of H2A (H2Aub) and H2B (H2Bub). The residues that are normally mono-ubiquitinated are: Lys-119 for H2A, and Lys-123 in yeast, or Lys-120 in vertebrate for H2B (Goldknopf et al. 1975, West and Bonner 1980). It has been reported that H3 and H4 and linker histone H1

could be ubiquitinated, but the biological function still has to be elucidated (Pham and Sauer 2000, Jason et al. 2002, Wang et al. 2006). The modifier enzymes responsible for ubiquitination are called histone ubiquitin ligases. The first to be identified was RING1B (E3 ubiquitin ligase), and it is responsible for Lys-119 H2A mono-ubiquitination. It belongs to the Polycomb group proteins so it is related with gene silencing, while on the contrary H2B ubiquitination has been related with gene transcription activation (Cao and Yan 2012). Different studies underline how histone ubiquitination and other histone modifications are interconnected. It has been described in literature how histone H2B mono-ubiquitination is required for subsequent H3K4 methylation and H3K79 methylation, all markers of active gene transcription (Dover et al. 2002, Sun and Allis 2002, Lee, Shukla et al. 2007). *BRCA1*, a known tumor suppressor gene, contains in its RING finger an E3 ubiquitin ligase and it can catalyze H2A mono-ubiquitination *in vivo* (Zhu et al. 2011). Inactivation of BRCA1 causes the development of breast and ovarian cancer. RNF20, the major H2B specific E3 ubiquitin ligase in mammals, is considered a putative tumor suppressor gene since its depletion can increase the expression of *c-myc* and *c-FOS*, two proto-oncogenes (Shema et al. 2008). Histone ubiquitination can be reversed by ubiquitin hydrolase. USP22 can remove ubiquitin from monoubiquitinated H2A and H2B (Zhang, Varthi et al. 2008, Zhao et al. 2008). Elevated expression of USP22 is related with poor prognosis in patients with breast cancer (Zhang et al. 2011). All of this evidence underlines the important role played by histone ligase/hydrolase for normal cell function and makes these enzymes "drug-able" targets for future cancer therapies.

The expression patterns of histone modifiers in human cancer suggest these genes are important in neoplastic transformation and have characteristic patterns of expression depending on tissue of origin, with implications for potential clinical application.

18.2.6 Epigenetic Readers

Readers typically provide an accessible surface (such as a cavity or surface groove) to accommodate a modified histone residue, and determine the modification (acetylation vs. methylation) or state specificity (such as mono- vs. tri-methylation of lysine) (Yun et al. 2011). The SRA (Set and Ring Associated) domain of Uhrf1 behaves likes a "hand" with two fingers, capable of flipping out the methylated cytosine with subsequent recruitment of DNMT1 (DNA methyltransferase 1) to methylate the cytosine of the newly synthetized DNA strand. Uhrf1 TTD (Tandem Tudor Domain) and PHD (Plant Homo Domain) are instead capable of recruiting respectively the histone methyltransferases Suv39H1 or G9a. Uhrf1 overexpression has been reported to be upregulated in various types of cancers, including breast, lung, pancreatic, astrocytomas, cervical, bladder cancer, retinoblastoma, and leukemia (Kofunato et al. 2012, Benavente et al. 2014, Alhosin et al. 2016). Hells (helicase, lymphoid specific, also known as lymphoid-specific helicase) is a putative chromatin remodeler belonging to the SWI/SNF subfamily that plays a central role at repetitive pericentromeric heterochromatin. Hells can remodel chromatin in order to render it accessible to DNA methyltransferase enzymes Dnmt3a or Dnmt3b, but not Dnmt1, and therefore supports *de novo* DNA methylation and stable gene silencing. Hells upregulation

has been described in human retinoblastoma and human prostate cancer (Benavente et al. 2014, von Eyss et al. 2012). BRD4 is a member of the bromodomain proteins of epigenetics readers. A bromodomain is an approximately 110 amino acid protein domain that recognizes acetylated lysine residues, such as those on the N-terminal tails of histones. The fusion of BRD4 with nuclear protein in testis (NUT) results in the development of NUT midline carcinoma (French et al. 2001). Downregulation of BRD7 is observed in hepatocellular carcinoma, and lower level of BRD7 expression is also used as an indicator of poor prognosis in patients with osteosarcoma (Chen et al. 2016). BRD8 somatic mutations have been reported in whole genome sequencing of human hepatocarcinoma (Fujimoto et al. 2012, Fujisawa and Filippakopoulos 2017). Selective inhibition of these epigenetic readers may be a novel tool for cancer treatment.

18.2.7 Epigenetic Mediators and Cancer

Epigenetics mediators are normally regulated in cancer by epigenetics modulators in order to increase pluripotency or survival. These genes are capable of counteracting proper maturation programs when ectopically expressed or overactive, and in order to do that, the mediators are capable of influencing the epigenetic states that define differentiated cell types. Large blocks of repressive H3K9me2 and H3K9me3 modifications along with DNA methylation coordinate the cell-type-specific repression of developmentally regulated genes. These areas are called large organized chromatin K9 modifications (LOCKs) and are largely absent from embryonic stem cells and cancer cell lines (Wen et al. 2009). Well-known pluripotency factors such as *NANOG* or *OCT4* (also known as *POU5F1*) and some WNT signaling members belong to this category (Feinberg, Koldobskiy, and Gondor 2016). *NANOG* is a transcription factor required for maintaining the pluripotency of embryonic stem cells and is not expressed in most normal adult tissues. However, several studies described NANOG overexpression on several tumors, including breast cancer. NANOG is not capable of inducing the mammary tumor alone, but when co-expressed with Wnt-1, promotes cell migration and invasion (Lu et al. 2014). OCT4 is another factor that plays a pivotal role as key regulator in pluripotency. It is believed that OCT4 maintains the pluripotency of spermatogonial (the earliest stage in the spermatocitic ontogeny) stem cells and keeps them in an undifferentiated, self-renewing state. Its aberrant expression may contribute to the neoplastic process in cancer cells (Gidekel et al. 2003). Moreover, the sex-determining Y-box (*SOX2*) gene, another important pluripotency factor, is amplified in small-cell lung cancer and squamous cell carcinomas of the lung and esophagus (Rudin et al. 2012, Bass et al. 2009). SOX2 is highly expressed in nasopharyngeal carcinoma, and lung adenocarcinoma (Luo et al. 2013, Chiou et al. 2010). NANOG and OCT4 overexpression are associated with increased metastatic potential in breast cancer and lung adenocarcinoma (Lu et al. 2014, Wang et al. 2014, Chiou et al. 2010). In conclusion *OCT4*, *NANOG*, and *SOX2* altered expression is capable of preventing proper maturation of the stem cells, and contributing to the development of different tumors. Finally, these genes could be potential markers of prognosis and a novel target of therapy for these tumors.

18.2.8 Epigenetic Modulators and Cancer

Epigenetic modulators are factors capable of influencing the activity of the epigenetics modifiers causing the destabilization of differentiation-specific epigenetic states. An example of epigenetic modulators is oncogenic *RAS* signaling. All RAS protein family members belong to a class of protein called small GTPase and are involved in transmitting signals within cells (cellular signal transduction). *KRAS* transformation, a member of the *RAS* genes, can drive the downregulation of TET enzymes (histone lysine demethylases described above) and this event increases DNA methylation, that facilitates the silencing of tumor suppressor genes (Wu and Brenner 2014). Moreover, about 70% of colorectal cancers with a *KRAS* mutation show chemical marks that "switch off" the expression of genes, known to suppress the growth of tumors. In actively growing human diploid fibroblasts, the *INK4A-ARF* locus is silenced by histone H3 lysine 27 tri-methylation (H3K27me3) directed by Polycomb group proteins. When such cells are exposed to cellular stress, such as oncogenic signals, the H3K27me3 mark on the locus is decreased, resulting in expression of *INK4A-ARF* tumor suppressor genes. KRAS can increase the level of the ZNF304 transcription factor that binds to the repressor complex made by KAP1-SETDB1-DNMT1. This event causes the lack of activation of the *INK4A-ARF* locus (Serra et al. 2014). Another example is *STAT3* (Signal transducer and activator of transcription 3) gene. This gene is an important regulator of *NANOG*, *OCT4*, and *SOX2* expression (all epigenetic mediators described above) (Do et al. 2013). STAT3 is also capable of promoting the acquisition of stem cell features in pancreatic cancer (Tyagi et al. 2016), so it can be speculated that external signals may lead to the activation of the epigenetic mediators through STAT3 activation. Moreover STAT3 is capable of interacting with epigenetic modifiers such as p300 histone acetyltransferase (HAT), or DNMT1 influencing gene expression and cell-type specific transcription (Hutchins, Diez, and Miranda-Saavedra 2013).

TP53, a tumor suppressor gene, is also capable of acting as an epigenetic modulator. *TP53* gain of function mutations can induce the expression of *MLL*1 and *MLL2* (mixed-lineage leukemia) genes, and this results in genome-wide increase in H3K4 trimethylation and gene transcription activation (Zhu et al. 2015). Moreover, mutated p53 can help in maintaining an open chromatin conformation at the *VEGFR2* promoter through the recruitment of the SWI/SNF complex (Pfister et al. 2015). Bi-allelic inactivation of *RB1* (retinoblastoma 1) gene, another important tumor suppressor, drives the development of human retinoblastoma, a pediatric tumor of the retina. Whole-genome sequencing of human retinoblastomas identified no genetic lesions in known tumor suppressor genes or oncogenes, other than *RB1*. Furthermore, the epigenetic profile showed profound changes compared to that observed in normal retinoblasts (Zhang et al. 2012). As for epigenetic mediators, the modulators are important targets for the cancer predisposing environment, and their mutations can lead to the destabilization of the epigenome.

18.2.9 microRNA and Cancer

MicroRNA (miRNAs) play an important role in regulating gene expression. MiRNA*s* can be classified as oncogenic, tumor suppressor, or context dependent

(Kasinski and Slack 2011). For example, miR-21 and miR-155 are frequently overexpressed in cancer, while miR-15~16 belong to the family of the onco-suppressor. MiR-146 instead is considered a context-dependent miRNA, because it may have opposing roles in tumorigenesis depending on the cellular context (Kasinski and Slack 2011). Interestingly it has been described in literature how miRNAs can regulate the expression of the epigenetic modifiers. Downregulation of miR-101 can lead to EZH2 (H3K27me3) overexpression in bladder and prostate cancer (Friedman et al. 2009, Varambally et al. 2008). Among the reported downregulated miRNAs in lung cancer, the miRNA miR-29 family (29a, 29b, and 29c) can target the 3'-UTRs of DNA methyltransferase DNMT3A and DNMT3B (*de novo* methyltransferases), two key enzymes involved in DNA methylation, that are frequently upregulated in lung cancer and associated with poor prognosis (Fabbri et al. 2007). In lung cancer miR-449 can downregulate HDAC1 expression, and this results in tumor suppression (Jeon et al. 2012, Rusek et al. 2015). Co-treatment with miR-449a and HDAC inhibitors had a significant growth reduction compared with HDAC inhibitor monotreatment. These results suggest that miR-449a/b may have a tumor suppressor function and might be a potential therapeutic candidate in patients with primary lung cancer. MiRNAs expression can be altered by epigenetic changes given that around half of the miRNA genomic sequences are associated with CpG islands (Weber et al. 2007). A good example is miR-127, which is embedded in a CpG island within a miRNA cluster. The expression of the whole cluster is downregulated or completely silenced in primary tumors (prostate, bladder and colon) and various cancer cell lines (HCT1116, HeLa and MCF7). Interestingly, miR-127 downregulation can be reversed using DNMT inhibitors (5-Aza-CdR) (Saito et al. 2006). MiR-9-1 and miR-34a/b are DNA hypermethylated in breast and colon cancer respectively (Lehmann et al. 2007, Toyota et al. 2008). MicroRNA also play a pivotal role in the early phase of cancer metastasis called epithelial-to-mesenchymal transition (EMT), characterized by the repression of E-cadherin. The zinc finger transcriptional repressors ZEB1, ZEB2, Snail1, and Twist1 are involved in E-cadherin regulation; and thus, EMT. The miR-200 family can inhibit ZEB in several cancer types such as breast, bladder, and ovarian cancers (Bendoraite et al. 2010, Gregory et al. 2008, Park et al. 2008, Adam et al. 2009). MiR-335 is also capable of suppressing migration/invasion through a different pathway involving the progenitor cell transcription factor SOX4 and extracellular matrix component Tenascin C (Tavazoie et al. 2008). MiR-34A is normally induced by the epigenetic modulator p53, while it can be repressed by the cytokine IL-6 and the oncogenic transcription factor STAT3. This event can promote EMT-mediated colorectal cancer invasion and metastasis (Rokavec et al. 2014). MiRNA-466 can reduce prostate cancer tumor growth and bone metastasis (Colden et al. 2017). The let-7 family is downregulated in several human cancers, which is thought to increase tumorigenicity and metastatic ability in breast cancer (Yu, Yao et al. 2007). Interestingly, the mature let-7 can be inhibited by another miRNA, miR-107, an event that promotes tumor progression and metastasis (Chen et al. 2011). Remarkably, circulating miRNAs may also serve as biomarkers for cancer prognosis. A study performed in serum samples from patients with colorectal cancer identified increased levels of circulating miR-92a and mir-29a (Huang et al. 2010).

Similarly, increased serum levels of miR-141 are observed in prostate cancer patients when compared to healthy individuals (Mitchell et al. 2008). The striking involvement of miRNA*s* in several critical cancer-associated processes makes them highly interesting molecules for therapeutic applications. So far, two potential approaches for the regulation of miRNA expression have been evaluated for their use in cancer treatment. One approach is to introduce antisense RNA (Anti-miRs), which can block the function of oncogenic miRNAs, or the re-introduction of a synthetic miRNAs to mimic the action of the tumor suppressor miRNAs. The other approach is focused on inducing the expression of the miRNAs using drugs. The use of drugs implies the modification of the oligonucleotide structure in order to avoid filtration by the kidneys and their clearance through the urine (Chan and Wang 2015).

18.2.10 Epigenetic Inactivation of DNA Repair Genes

Efficient DNA repair is crucial for preventing cancer. Earlier, we discussed how mutations in DNA repair genes could cause inherited cancer syndromes. We also examined how mutations in the epigenetic machinery contribute to cancer. Additionally, DNA repair pathways may be inactivated or decreased in effectiveness by epigenetic inactivation mechanisms affecting DNA repair genes. While mutations in the DNA repair machinery are quite rare in sporadic cancers, those that present with DNA repair deficiencies have one or more epigenetic alterations that reduce or silence the expression of the DNA repair genes (Bernstein and Bernstein 2015). DNA methylation at the promoter region of genes participating in DNA repair pathways including DR, BER, NER, HR, NHEJ, and others has been reported in several cancers, summarized in Table 18.3 and discussed below. It can be assumed that the epigenetic inactivation of DNA repair genes can result in an increase in genetic instability that contributes to tumor progression. On the other hand, diminished DNA repair may

TABLE 18.3
DNA Repair Genes Methylated in Cancer

DNA Repair Pathway	Gene Methylated
BER	MBD4 (Howard et al. 2009, Peng et al. 2006); TDG (Peng et al. 2006); OGG1 (Guan et al. 2008); NEIL1 (Do et al. 2014)
DR	MGMT (Herfarth et al. 1999)
NER	XPC (Yang et al. 2010); RAD23A (Peng et al. 2005); RAD23B, ERCC1 (Chen et al. 2010); ERCC4
MMR	MLH1 (Guan et al. 2008, Esteller et al. 1998, Wang et al. 2003, Kim et al. 2010, Hinrichsen et al. 2014); MSH2 (Wang et al. 2003, Lawes et al. 2005, Hinrichsen et al. 2014); MSH3 (Kim et al. 2010), MSH6 (Lawes et al. 2005); PMS2 (Hinrichsen et al. 2014)
HR	BRCA1 (Dobrovic and Simpfendorfer 1997, Lee, Tseng et al. 2007)
NHEJ	XRCC5 (Lee, Tseng et al. 2007)

also lead to reduced cell survival in general, and additional events are likely occurring that enable a cell with reduced repair capacity to undergo uncontrolled proliferation instead of cell death (e.g. *TP53* pathway inactivation).

18.2.10.1 DR

Epigenetic silencing of the *MGMT* gene has been broadly reported in solid tumors including colon cancer (Herfarth et al. 1999), glioblastoma (Esteller et al. 2000), non-small cell lung cancer (Wolf et al. 2001), and gastric cancer (Oue et al. 2001), among others. Furthermore, in glioma patients, epigenetic silencing of the *MGMT* gene correlates with better response to alkylating agent treatments when compared to patients with tumors with active *MGMT* (Esteller et al. 2000, Hegi et al. 2005).

18.2.10.2 BER

As discussed previously, in BER, MBD4 and TDG are important enzymes for counteracting the hydrolytic deamination of 5-methylcytosine. Promoter methylation of these two DNA repair genes has been observed in various cancer cell lines (Peng et al. 2006, Howard et al. 2009). Furthermore, epigenetic silencing of OGG1, involved in the repair of 8-oxoguanine, has also been observed in some cancer cell lines (Guan et al. 2008).

18.2.10.3 NER

The *XPC* gene, which encodes for the essential subunit for the damage recognition complexes in GGR (Friedberg 2001, Riedl, Hanaoka, and Egly 2003), is silenced in bladder cancer through DNA hypermethylation (Yang et al. 2010). In addition, *RAD23A* and *ERCC1*, two genes that are involved in DNA damage recognition and incision, respectively, as part of the NER pathway are also inactivated through DNA methylation of their promoter region. The *RAD23A* gene is methylated in the multiple myeloma cell line KAS-6/1 (Peng et al. 2005) and *ERCC1* is epigenetically silenced through DNA methylation is associated with drug resistance in glioma cell lines and glioma tumors (Chen et al. 2010).

18.2.10.4 MMR

Approximately 13% of all colorectal cancers present deficiencies in MMR. Among the majority of these—particularly in sporadic disease—have loss of MMR due to silencing of *MLH1* through DNA methylation of the promoter region of the gene (Truninger et al. 2005, Kane et al. 1997). Additionally, this gene is epigenetically inactivated in other types of cancer, including sporadic endometrial carcinoma (Esteller et al. 1998), gastric cancers (Fleisher et al. 1999), ovarian tumors (Gras et al. 2001, Zhang, Zhang et al. 2008), oral squamous cell carcinoma (Czerninski et al. 2009), neck squamous cell carcinoma (Liu et al. 2002), and acute myeloid leukemia (AML) (Seedhouse, Das-Gupta, and Russell 2003). Beyond *MLH1*, other genes that belong to the MMR pathway are also controlled by promoter methylation, including *MSH2*, *MSH3*, and *MSH6*. Indeed, *MSH2*, *MSH3* and *MSH6* are also DNA methylated in colorectal cancer (Lawes et al. 2005, Benachenhou et al. 1998). MSH2 is also DNA methylated in primary non-small cell lung cancer (Wang and Qin 2003), oral squamous cell carcinoma (Czerninski et al. 2009), and ovarian cancer

(Zhang, Zhang et al. 2008). Interestingly, the methylation frequencies in *MLH1* and *MSH3* were significantly higher in elderly gastric carcinoma patients than in younger patients (Kim et al. 2010). Thus, DNA methylation of these genes may have considerable importance in cancer development and as a prognostic factor.

18.2.10.5 HR

Mutation of the *BRCA1* tumor suppressor gene is an important contributing factor in hereditary breast and ovarian cancer. However, *BRCA1* mutations have not been detected in the sporadic forms of these cancers. Still, BRCA1 mRNA and protein levels are reduced in some sporadic breast and ovarian cancers. Aberrant promoter methylation of the *BRCA1* promoter is correlated with the low mRNA and protein levels (Dobrovic and Simpfendorfer 1997). The *BRCA1* promoter is also methylated in gastric cancer (Bernal et al. 2008), non-small cell lung cancer (Lee, Tseng et al. 2007), uterine leiomyosarcoma (Xing et al. 2009), and bladder cancer (Yu, Zhu et al. 2007).

18.2.10.6 NHEJ

While genetic mutation of the genes that regulate NHEJ have not been reported, a deficiency in expression of the Ku80 protein has been observed in melanoma (Korabiowska et al. 2002). In addition, low expression of Ku80 was found in 15% of adenocarcinoma type and 32% of squamous cell type non-small cell lung cancers, which correlated with hypermethylation of the *XRCC5* promoter (Lee, Tseng et al. 2007).

18.2.11 Epigenetics in Cancer Stem Cells

Cancer stem cells (CSCs) are a rare subpopulation of cells present within tumors with the capacity to self-renew and regenerate the whole tumor (Reya et al. 2001). While CSCs were first described in myeloid leukemia, they have also been identified in solid tumors including breast, prostate, colon, brain, pancreas, liver, ovary, melanoma, skin, and head and neck. CSCs are thought to be responsible for sustaining tumor growth and metastases. Most importantly, CSCs are more resistant to therapeutic agents than the non-stem tumor cells, suggesting that CSCs are responsible for tumor relapse. Most of the epigenetic mechanisms with roles in promoting the acquisition of uncontrolled self-renewal previously described in this chapter may contribute to CSC formation and maintenance. One of the best examples of the relevance of DNA methylation in CSC regulation and tumor growth is portrayed in leukemia stem cells, where abrogation of Dnmt1 expression blocks leukemia development in a mouse model. Furthermore, haploinsufficiency of Dnmt1 results in tumor suppressor gene activation, impaired CSC self-renewal and delayed progression of leukemogenesis (Trowbridge et al. 2012). Histone methylation also appears to be involved in CSC formation and maintenance. The Polycomb group complexes target similar sets of CpG-containing genes in embryonic stem cells as in cancer cells, suggesting that these genes may be responsible for the emergence of the CSC phenotype during tumorigenesis (Schlesinger et al. 2007, Widschwendter et al. 2007, Ohm et al. 2007). Further, a knock-in mouse model inducing the expression of EZH2 in hematopoietic stem cells was shown to promote myeloid expansion, which indicates a stem cell-specific EZH2 oncogenic role in myeloid disorders (Herrera-Merchan et al. 2012).

Several other studies support the idea that increased EZH2 expression in some tumors contributes to the maintenance of a reversible and undifferentiated stem-like phenotype in cancer cells and the expansion of breast CSCs (Chang et al. 2011, Burdach et al. 2009). Inhibition of other Polycomb proteins including LSD1 and MLL1 has also been shown to decrease CSC proliferation potential and tumorigenicity (Wang, Lu et al. 2011, Heddleston et al. 2012). Several miRNAs cooperate with Polycomb complexes and DNA methylation to regulate the balance between self-renewal and differentiation in CSCs (Esquela-Kerscher and Slack 2006; Volinia et al. 2006). Let-7 is thought to play a critical role in the breast CSC maintenance (Viswanathan et al. 2009; Yang et al. 2010) and contributes to EZH2 overexpression in prostate cancer (Kong et al. 2012). The downregulation of the miR-200 family members is linked to proliferation of CSCs and their ability to form tumors (Iliopoulos et al. 2010; Lo et al. 2011; Shimono et al. 2009). Further, miR-34a, which is underexpressed in CSCs, negatively regulates the tumor initiating capacity of prostate (Liu et al. 2011), pancreatic (Ji et al. 2009b), and breast (Yu et al. 2012) CSCs.

18.3 CONCLUSION AND FUTURE PROSPECTS

The ongoing molecular characterization of DNA damage response and repair pathways, and how they influence chemo- and radio-resistance, guides the development of novel cancer therapeutics. This information is instrumental in developing DNA repair inhibitors, the latest effort in creating more targeted anticancer treatments that cause less toxicity to normal cells. DNA repair inhibitors are essential in the application of synthetic lethal combinations of drugs and genetic deficiencies.

It is interesting to point out that while in most repair pathways there are several proteins involved in the repair process, within each DNA repair pathway there are specific genes that are preferentially epigenetically silenced. It is yet to be determined whether this specificity is due to selection of particular repair gene silencing events in promoting tumorigenesis or is due to preferential targeting of the DNA methylation machinery to specific DNA repair gene promoters (Lahtz and Pfeifer 2011).

It is also interesting that epigenetic inactivation of DNA repair pathways can lead to different clinical outcomes. Reduced repair capacity for alkylated guanines by promoter methylation of the *MGMT* gene provides a therapeutic benefit in patients with glioma (Esteller et al. 2000). On the other hand, inactivation of the MMR system is associated with resistance of cells to cisplatin treatment (Fink, Zheng et al. 1997, Fink, Nebel et al. 1997). With the mounting knowledge regarding the epigenome of specific cancer types, there is now an opportunity to develop chemotherapy regimens tailored to a patient's DNA repair gene status by incorporating information on epigenetic silencing of the relevant genes in the tumor.

Studies during the last decade emphasize the importance of epigenetic mechanism at most stages during cancer development, which given the reversible nature of epigenetics, present novel opportunities for therapeutic intervention. The observation that genes involved in epigenetic regulation are among the most commonly mutated gene families, underscores the importance of further understanding the epigenetic mechanisms participating in tumor maintenance and progression. Advances in the

field will likely require elucidating how different epigenetic proteins contribute to gene-specific gene expression modulation, which would refine our understanding of their roles in tumor maintenance. Considering the multiple roles of epigenetics in cancer, and the possible interference with normal homeostasis, epigenetic interventions in carcinogenesis still faces many challenges, but also offers groundbreaking opportunities to the treatment of these devastating malignancies.

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