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The Emerging Role of Epigenetic Modifiers in Repair of DNA Damage Associated with Chronic Inflammatory Diseases

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Abstract

At sites of chronic inflammation epithelial cells are exposed to high levels of reactive oxygen species (ROS), which can contribute to the initiation and development of many different human cancers. Aberrant epigenetic alterations that cause transcriptional silencing of tumor suppressor genes are also implicated in many diseases associated with inflammation, including cancer. However, it is not clear how altered epigenetic gene silencing is initiated during chronic inflammation. The high level of ROS at sites of inflammation is known to induce oxidative DNA damage in surrounding epithelial cells. Furthermore, DNA damage is known to trigger several responses, including recruitment of DNA repair proteins, transcriptional repression, chromatin modifications and other cell signaling events. Recruitment of epigenetic modifiers to chromatin in response to DNA damage results in transient covalent modifications to chromatin such as histone ubiquitination, acetylation and methylation and DNA methylation. DNA damage also alters non-coding RNA expression. All of these alterations have the potential to alter gene expression at sites of damage. Typically, these modifications and gene transcription are restored back to normal once the repair of

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the DNA damage is completed. However, chronic inflammation may induce sustained DNA damage and DNA damage responses that result in these transient covalent chromatin modifications becoming mitotically stable epigenetic alterations. Understanding how epigenetic alterations are initiated during chronic inflammation will allow us to develop pharmaceutical strategies to prevent or treat chronic inflammation-induced cancer. This review will focus on types of DNA damage and epigenetic alterations associated with chronic inflammatory diseases, the types of DNA damage and transient covalent chromatin modifications induced by inflammation and oxidative DNA damage and how these modifications may result in epigenetic alterations.

Keywords: chronic inflammation, oxidative DNA damage, DNA repair, epigenetic alterations.

1. Introduction

In 1863 Rudolf Virchow observed that “lymphoreticular cells were infiltrated” into neoplastic tissues of malignant tumors and hypothesized that there is a link between tissue inflammation and tumor development [1]. Today, it is clear that chronic inflammation is a risk factor for tumorigenesis, and epidemiological studies show that more than 15% of cancer cases are associated with chronic inflammation [2]. Many studies suggest that tumorigenesis is associated with infections, dietary/lifestyle choices, obesity, type 2 diabetes, environmental exposures, chemical inhalation and tobacco use. The fundamental basis that underlies all of these factors is chronic inflammation. Hence, chronic inflammation can be viewed as a link that connects many processes to tumor initiation and development [3]. Two major pathways (extrinsic and intrinsic) have been identified that connect inflammation to cancer [4]. In the extrinsic pathway, the inflammatory microenvironment in which epithelial cells are exposed to cytokines, chemokines and altered signal transduction cascades facilitates tumor initiation and development. In the intrinsic pathway, inflammation-induced accumulation of genetic and epigenetic alterations and functional cooperation between these changes promote malignant transformation of the epithelial cell.

Reactive oxygen species (ROS) are a group of highly reactive free radicals that are derived from oxygen and have a role in cell signaling and inflammation. It is known that overproduction of ROS acts as an important mediator of the causative relation between inflammation and cancer [5]. Respiration bursts by leukocytes release large amounts of ROS at sites of inflammation. ROS can have extrinsic effects such as inducing cell proliferation as well as intrinsic effects such as inducing DNA damage. ROS-induced DNA damage such as base damage, single strand breaks (SSBs) and double strand breaks (DSBs) may result in genetic mutations, which increase the risk of tumor initiation and progression.

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In addition to causing genetic alterations, ROS can also promote epigenetic changes that play a role in cancer. Epigenetic alterations are mitotically heritable gene expression changes that are not due to changes in the DNA sequence. DNA is packaged into repeating units called nucleosomes, which are comprised of DNA wound around eight histone proteins. These nucleosomes coil together and become tightly packed to form chromatin. Post-translational modifications to histone tails can induce either chromatin condensation or relaxation, depending on the specific mark, and such modifications can result in altered gene expression. DNA methylation is a covalent modification of DNA that when present in gene promoters can cause transcriptional repression. While not considered a traditional epigenetic alteration, non-coding RNAs (ncRNAs) are functional RNA molecules that are transcribed from DNA and regulate gene expression at the transcriptional and post-transcriptional level. A well-studied class of ncRNAs is microRNAs (miRNAs), which are smaller than 22 nucleotides and function in post-transcriptional gene silencing. Histone modifications, DNA methylation, and ncRNA expression are three major types of alterations that have been demonstrated to change at sites of chronic inflammation and each plays important roles in cancer initiation and development [6] [7]. For the purpose of this review, we use the term “epigenetic modifiers” to describe proteins that directly modify the epigenome through chromatin modifications and changes in the expression of ncRNAs even if these changes are not mitotically heritable.

While the links between inflammation, oxidative DNA damage, epigenetic alterations, and cancer are well established, the mechanism by which inflammation causes cancer-specific epigenetic alterations is not completely understood. Here, we provide evidence from the literature that transient histone tail modifications, DNA methylation, and ncRNA expression changes initiated during repair of DNA damage are potential mechanisms for the initiation of epigenetic alterations in inflammation-induced cancer. In this review, we will first discuss the types of DNA damage and epigenetic alterations associated with specific inflammatory conditions. Then, we will discuss types of DNA repair mechanisms initiated by oxidative damage and the chromatin and ncRNA alterations that occur during these DNA repair processes. We will conclude with evidence that such repair-induced chromatin changes may result in heritable epigenetic transcriptional silencing of genes.

2. Types of DNA damage and epigenetic alterations associated with inflammatory diseases.

There is a broad spectrum of agents that can initiate inflammatory diseases. Here, we discuss several common causes of chronic inflammation as examples, including (1) infectious agents, such as bacteria, parasites, and viruses, (2) non-infectious agents and disease conditions, including chemicals from the environment, tobacco, drinks or food, and diseases such as obesity and type 2 diabetes. Leukocytes and mast cells are recruited to sites of damage induced by these sources to engulf the bacterium, parasites, viruses, chemical particles, or debris from dead/dying cells. During this process, leukocytes produce large amounts of reactive oxygen species (ROS) to damage and degrade these internalized agents. Additionally, they release cytokines and chemokines to recruit more immune cells to the site of inflammation. Usually, this response ends after the hazardous agents or particles are

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destroyed. However, when these sources persist, they can lead to accumulation of oxidative DNA damage and result in neoplastic transformation of epithelial cells at sites of inflammation, which may increase one's risk of developing cancer. Chronic inflammatory diseases are also characterized by epigenetic changes, including altered histone modifications, DNA methylation, and ncRNA expression. While there is limited direct evidence, it is hypothesized that inflammation induces these epigenetic alterations that then contribute to disease formation. We will discuss several examples of causes of chronic inflammation and the evidence available that implicates a causal relationship between inflammation-induced oxidative damage and epigenetic alterations (see also Table 1).

2.1 Chronic inflammation caused by infectious agents

Infection with certain organisms has been associated with epigenetic alterations, DNA damage and the development of cancer. *Helicobacter pylori (H. pylori)* infection is associated with chronic inflammation of the stomach (gastritis) and increased risk of gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma [8]. At the molecular level, *H. pylori* infection in the stomach is known to induce DNA base damage and double strand breaks (DSBs) [9]. *H. pylori* infection is also associated with genome-wide DNA hypomethylation [10] [11], global de-phosphorylation of the histone residue H3Ser10 and de-acetylation of the histone residue H3K23 [12], and upregulation of oncogenic miRNAs such as miRNA-21, miRNA-222 and miRNA-223 and downregulation of tumor suppressive miRNAs such as miRNA-185, miRNA-204 and miRNA106b. [13]. Importantly, oxidative damage-associated with *H. pylori* infection is necessary for the associated DNA methylation changes [14]. Infection with the parasitic worms called Schistosoma or blood-flukes is linked to the disease schistosomiasis, which involves increased oxidative DNA base damage [15], increased DNA methylation at the promoter region of tumor suppressor genes such as *Ras association domain family 1 isoform A (RASSF1A)* and *Tissue inhibitor metalloproteinase 3 (TIMP3)* [16], increased miRNA expression such as miRNA-223 and decreased miRNA expression such as miRNA-454 [17], and formation of bladder, hepatocellular and rectal carcinomas [18]. Hepatitis B and C viruses are well-known etiological agents for hepatitis and they are associated with increased risk of cirrhosis and hepatocellular carcinoma [19]. Hepatitis viral infection causes high level of ROS-induced DNA base damage [20], increased DNA methylation at the promoter of tumor suppressor genes including *Cyclin-dependent kinase inhibitor 2A (CDKN2A, or p16), E-cadherin (CDH1),* and *Insulin-like growth factor binding protein 3 (IGFBP-3),* decreased promoter DNA methylation of tumor promotion-related genes such as *Cadherin-6 (CDH6)* and *Aldehyde dehydrogenase 1 (ALDH1)* [21], increased tri-methylation of the histone residue H3K4 at the promoter of the oncogene *Myc proto-oncogene protein* (*C-MYC),* and decrease of the histone marks, H4R3me2, H4K16Ac and H4K20me3 at the promoter of tumor suppressor genes such as *Phosphatase and tensin homolog (PTEN), Epidermal growth factor receptor (EGFR),* and *IGFBP3* [22] [23]. Hepatitis viral infection also regulates the expression of miRNAs. For instance, upon infection, miRNA-602 is upregulated and thereby its downstream target-the tumor suppressor gene *RASSF1A* is inhibited, whereas miRNA-152 is

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downregulated and its target-the epigenetic protein *DNA methyltransferases 1 (DNMT1)* is upregulated [24]. Infection with the gram-negative bacterium *Neisseria gonorrhea* or *Chlamydia* is associated with elevated ROS and DSBs [25] [26], global increase of the histone mark H3K9me3 [26], and increased expression of miRNAs that are known to inhibit inflammation, including miRNA-155, miRNA-142-3p, miRNA-174-3p, miRNA-105 and miRNA-132 [27]. *Neisseria gonorrhea* or *Chlamydia*-caused infections are also associated with pelvic inflammatory diseases [28] and ovarian carcinoma [29]. Human papillomaviruses are risk factors for cervical carcinoma [30] and are associated with oxidative DNA base damage and DSBs [31] as well gain of aberrant DNA methylation at the promoter of tumor suppressor genes including *CDH1*, *Interferon-κ (INF-κ)* and *telomerase reverse transcriptase (hTERT)* [32], and reduced the expression of miRNAs that can act as tumor suppressors such as miRNA-145 [33] [34]. Enterotoxigenic *Bacteroides fragilis* (ETBF) is a strain of *B. fragilis* that secretes a 20-kDa metalloprotease toxin. ETBF is an etiological agent of diarrheal disease in humans that is associated with colitis and colorectal cancer [35], increased ROS, oxidative base damage and DSBs [36] [37], Acute ETBF infection is associated with increased binding of DNMT1 and Enhancer of zeste homolog 2 (EZH2) to chromatin and to the promoters of tumor suppressor genes [37] [38]. ETBF-induced tumors have increased DNA methylation at 203 CpG island regions including the promoter CpG islands of putative tumor suppressor genes *Homeobox a5 (Hoxa5)*, *Polymerase gamma (Polg)*, and *TNF receptor superfamily member 13B (Tnfrsf13b*) [37]. Overall, these examples suggest that inflammation induced by infectious agents is strongly causally associated with oxidative DNA damage and epigenetic alterations.

2.2 Chronic inflammation caused by non-infectious agents and diseases

Several noninfectious agents have been demonstrated to be associated with chronic inflammation, increased cancer risk, DNA damage, and epigenetic alterations. For instance, repetitive and excessive reflux of gastric acid into the esophagus due to a dysfunctional lower esophageal sphincter can lead to inflammatory conditions such as reflux esophagitis or Barrett's esophagus that subsequently may develop to esophageal carcinoma [31]. Gastric acid, bile acid salts, and proteases in pancreatic juices have been shown to play roles in damaging the esophagus mucosa, activating ROS-producing enzymes and inducing chronic inflammation [39, 40]. Barrett's esophagus is associated with high levels of ROS and DSBs [39, 40], gain of DNA methylation at the promoter region of tumor suppressor genes such as *Adenomatous polyposis coli (APC)*, *TIMP3*, and *hTERT*, reduced genome-wide DNA methylation [41], upregulation of tumor-promoting miRNAs such as miRNA-21 and miRNA-25, and downregulation of tumor suppressing miRNA such as miRNA-205 and miRNA-203 [42, 43].

Occupational and environmental exposure to airborne asbestos fibers and silica particles that come from soil, sand, rocks and many other minerals gives rise to pleural and lung fibrosis as well as chronic inflammatory conditions such as asbestosis and silicosis, which are linked to increased risk of mesothelioma and lung carcinoma [44]. Asbestos fibers and silica particles can induce high level of ROS production by the Fenton's reaction that is initiated by the iron at their surfaces [45, 46] as well as by

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their ability to cause incomplete phagocytosis and inflammation [47]. Increased production of ROS [48], base oxidation damage [49], single strand breaks (SSBs) [49], DSBs [50], as well as gain of aberrant DNA methylation at the promoter of the tumor suppressor genes *O^6-methylguanine-DNA methyltransferase (MGMT), p16, RASSF1A*, and *PTEN* [51] [52] [53], and reduced expression of tumor suppressing miRNAs, miRNA-181 and miRNA-29 [54] are caused by asbestos and/or silica particles.

Cigarette smoking commonly causes chronic obstructive pulmonary disease (COPD) , a risk factors for lung cancer [55]. Elevated leukocyte recruitment and aberrantly produced cytokines in COPD contribute to ROS production, creating a pro-tumorigenic microenvironment and inducing lung tumorigenesis [56]. Some components of tobacco also directly produce ROS. For instance, benzo[a]pyrene can be metabolized to quinone, which can produce ROS through redox reactions. Other components may induce ROS by triggering inflammatory responses [57]. Cigarette smoking contributes to oxidative DNA damage including base damage [58], SSBs [59], and DSBs [60]. Cigarette smoking is associated with global alteration of CpG methylation sites and broad changes in gene expression, even after many years after smoking cessation in human population studies [61]. In human bronchial epithelial cells, cigarette smoke condensate induces hypermethylation at promoter CpG sites of tumor suppressor genes and hypomethylation at the global scale [62]. This study also demonstrated that the active transcriptionally-active histone marks H4K16Ac and H4K20me3 decrease genome-wide, while the transcriptionally-repressive histone mark H3K27me3 increases globally after cigarette smoke condensate administration [63]. Interestingly, a recent study demonstrated that chronic cigarette smoke condensate exposure initially induces increased chromatin binding of epigenetic proteins, including DNMT1, EZH2, and Sirtuin 1 (SIRT1), later manifesting in aberrant DNA hypermethylation at promoter CpG islands by 10 months of exposure [64]. These findings suggest that binding of epigenetic modifiers to chromatin plays an important role in initiating aberrant DNA methylation in response to cigarette smoking. Smoking also downregulates the expression of the tumor suppressing miRNAs, miRNA-218, miRNA-15a, miRNA-199b, and miRNA125b [65] [66].

Heavy alcohol consumption is positively associated with increased risk of chronic pancreatitis and liver cirrhosis [67, 68], which in turn increases the risk of pancreatic and liver cancer, respectively [68] [69]. Ethanol induces elevated production of ROS via alcohol dehydrogenase catalyzed alcohol metabolism [70] and causes oxidative base damage [71]. Excessive alcohol consumption also induces the formation of acetaldehyde and induces DNA adducts such as acetaldehyde-DNA adduct and *N2*-ethyl-2′-deoxyguanosine, which may induce activation of multiple repair pathways including base excision repair (BER) and the Fanconi anemia-breast cancer susceptibility (FA-BRCA) DNA damage response network [72] [73]. Chronic alcohol consumption is associated with reduction of global DNA methylation in hepatic cells [74] and colonic mucosa in rats [75]. In addition, this chronic alcohol consumption-induced DNA hypomethylation is heritable through the male germ line [76]. On histone H3, ethanol treatment induces increased acetylation, decreased di-methylation of the histone residue Lys9 (H3K9me2),

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decreased di-methylation of Lys4 (H3K4me2), and increased phosphorylation of serine 10 and serine 28 (H3Ser10ph and H3Ser28ph) globally in hepatocytes in vitro [77] [78]. Chronic ethanol feeding leads to change of miRNAs that are involved in lipid metabolism, iron and glucose maintenance, and liver regeneration. (reviewed in [79]).

Obesity is a condition where excessive body fat accumulation impairs a person's health and fitness. Type 2 diabetes is a chronic metabolic disease that involves impairment of a person's insulin sensitivity and glucose metabolism. Chronic inflammation plays an important role in linking obesity and type 2 diabetes to the development of cancer [80, 81]. Elevated levels of pro-inflammatory factors and ROS and reduced anti-oxidative capacity in individuals with obesity and type 2 diabetes contributes to tumor initiation and development [82]. Adipose tissue in people with these conditions is highly inflamed with enrichment of immune cells, production of high level of ROS and secretion of numerous pro-inflammatory factors. The pro-inflammatory factors, along with ROS can enter peripheral blood to promote systemic inflammation, which is implicated in the emergence of many metabolic disorders [83]. Systemic inflammation induces DNA damage including DSBs and base oxidation [84, 85]. Obesity is shown to be associated with alteration in DNA methylation of individual candidate genes in blood leukocytes and in adipose tissue. For instance, reduced DNA methylation at the promoter of *Tumor necrosis factor alpha (TNF-α)* and increased methylation at the promoter of *Aryl hydrocarbon receptor nuclear translocator-like (ARNTL or BMAL1)* are found in blood leukocytes in obese compared to lean individuals. Type 2 diabetes is associated with hypermethylation at the promoter of the genes that are involved in cell growth and energy metabolism such as *Cyclin dependent kinase inhibitor 2A (CDKN2A)*, *Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A)*, and *Pancreatic and duodenal homeobox 1 (PDX1)* [86] [87]. Obesity and type 2 diabetes are associated with increased global levels of the histone marks H3K9me3 and H3K27me3, and decreased acetylation of the histone residue H3K14 at the promoter of metabolism-associated genes in liver [88]. Obesity and type 2 diabetes are associated with deregulation of miRNAs which are involved in many biological processes linked to cancer, including cell proliferation, lipid/glucose metabolism, adipocyte differentiation, insulin signaling and appetite regulation. For instance, downregulation of miRNA-143 contributes to the development of obesity-induced early onset type 2 diabetes and cancer through deregulation of glucose metabolism, apoptosis and metastasis. [89]

3. The types of DNA damage and DNA repair pathways induced by ROS produced at sites of inflammation.

Macrophages and lymphocytes infiltrate tissue at sites of chronic inflammation. Macrophages are phagocytes capable of recognizing and digesting bacteria, viruses, foreign particles, cancer cells and anything without an antigen. They produce proteases, antimicrobial peptides, lysozyme and most importantly high level of ROS. It is estimated that phagocytes can produce ROS at a rate of 0.5 mM/sec [90]. ROS produced in phagosomes are capable of quickly damaging the DNA of internalized foreign microbes and causing death of microbial cells. However, ROS such as hydrogen peroxide (H_2O_2) may

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diffuse freely across cell membranes to damage the DNA of host cells as well [91, 92], particularly the epithelial cells present in mucosal surfaces. Long-term exposure to ROS may result in persistent DNA damage of the host's epithelial cells.

Several ROS family members are involved in inflammation including superoxide anion ($O2^{\bullet-}$), H_2O_2, peroxynitrite ($ONOO^-$), hydroxyl radical ($OH^\bullet$), and hypochlorous acid (HOCl) [93]. $O2^{\bullet-}$ is primarily produced via electron leakage in the mitochondrial respiration chain and via NADPH oxidase activation. Dismutation of $O2^{\bullet-}$ may occur spontaneously or by the enzyme superoxide dismutase (SOD) to produce H_2O_2. Both $O2^{\bullet-}$ and H_2O_2 themselves are poorly reactive, however, they can be converted to highly reactive and toxic species such as $OH^\bullet$ and $ONOO^-$ that may cause DNA damage. For instance, $O2^{\bullet-}$ and H_2O_2 yield hydroxyl radicals ($OH^\bullet$) via Haber-Weiss and Fenton reactions, respectively [94] and $OH^\bullet$ cause base modifications and damage, SSBs and DSBs, DNA-protein crosslinks, and abasic sites [95]. $O2^{\bullet-}$ also interacts with nitric oxide (NO) to generate $ONOO^-$. Base damage, abasic sites, and SSBs are caused by exposure to $ONOO^-$ [96]. Myeloperoxidase (MPO) can interact with H_2O_2 to oxidize halides leading to production of HOCl, which induces base modifications and strand breaks [97]. The majority of DNA damage that is induced by ROS is base damage and SSBs [98]. We will briefly discuss the major types of DNA damage induced by ROS exposure and the repair pathways for those types of damage.

3.1 ROS causes base damage that is repaired by BER or non-canonical mismatch repair (MMR).

Among approximately 100 types of base oxidation that have been identified in model studies, 8-oxoguanine (8-oxo-G) is the most abundant form of base damage during oxidative stress [99] and inflammation [100], possibly because all of the above-described species, including H_2O_2, $O2^{\bullet-}$, $OH^\bullet$, $ONOO^-$ and HOCl can convert guanine to 8-oxo-G [101]. Increased 8-oxo-G is associated with mutagenesis and carcinogenesis [99] and can be repaired by BER or non-canonical MMR. Single 8-oxo-G base damage is repaired by short-patch BER with the DNA glycosylase Oxoguanine glycosylase 1 (OGG1) recognizing and removing 8-oxo-G. This removal of the damaged base leaves an abasic site (AP-site). This AP-site is repaired by short-patch SSB repair (described below). However, tandem 8-oxo-G damage can be refractory to repair by OGG1 [102]. MMR proteins MSH2 and MSH6 play important roles in repairing clustered oxidative damage in a cell cycle-independent fashion [103]. In this non-canonical MMR pathway, the MSH2 and MSH6 heterodimer first recognizes and interacts with clustered oxidized DNA lesions, followed by recruitment of mono-ubiquitinated Proliferating cell nuclear antigen (PCNA) and Polymerase η (Pol η) to complete the DNA repair [103].

3.2 ROS causes single or double strand breaks and initiates SSB or DSB repair.

$OH^\bullet$ produced from H_2O_2 can induce SSBs and DSBs directly by removing a hydrogen atom from the sugar in the DNA backbone to induce a ruptured ribose ring and breakage of the strand [104]. DSBs are the most toxic form of DNA damage to cells because DSBs may interrupt or arrest many important cellular processes such as transcription, replication, and

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recombination. DSBs are indirectly formed from SSBs and base damage when replication machinery fails to replicate damaged DNA causing replication forks to eventually collapse, when transcription machinery encounter SSBs and base damage, or when multiple SSBs or base damage occur in close proximity [98]. SSBs and DSBs are repaired by well-studied mechanisms that we will briefly review below to introduce key repair proteins.

A SSB is quickly detected by the sensor Poly (ADP-ribose) polymerase-1(PARP-1). Depending on the length of the breakage, the SSB can be processed and repaired by either short-patch SSB repair (1 nucleotide) or long-patch SSB repair (2-12 nucleotides). Both short patch and long-patch SSB repair involve three major steps: termini end processing (by enzymes such as Apurinic/apyrimidinic endonuclease 1 (APE1)), gap filling (by polymerases), and ligation (by ligases) [105].

A DSB is recognized by the MRN complex (MRE11/RAD50/NBS1, Meiotic recombination 11 homolog 1-RAD50- nibrin1) and activates the kinase ATM (Ataxia telangiectasia mutated). Activated ATM then induces the sequential recruitment of DNA damage repair proteins to the sites of damage and activates checkpoint proteins to arrest the cell cycle in order to facilitate repair. In response to DSBs, ATM phosphorylates histone variant H2AX and phosphorylation of H2AX (γH2AX) recruits other repair proteins to the damage sites. One of the proteins that γH2AX recruits is Mediator of DNA damage checkpoint 1 (MDC1) [106], which is a scaffold protein that allows many other subsequent chromatin remodeling proteins and DNA repair proteins to bind the chromatin at the site of damage [107]. Recruitment of MDC1 by γH2AX is biologically important because it not only initiates chromatin structure alterations for repair protein accession but also creates positive feedback that spreads γH2AX at sites of damage [108]. Depending on the cell cycle, DSBs can be repaired by two pathways, homologous recombination (HR) or non-homologous-end joining (NHEJ). HR is an S and G2 phase-dependent repair pathway whereas NHEJ is cell cycle-independent. Both HR and NHEJ have four major repair steps: recognition, recruitment, end processing, and repair [106].

4. The role of epigenetic modifiers in DNA damage repair

As mentioned above, DNA repair proteins are recruited and interact with damaged DNA in response to ROS. However, the architecture of nucleosomes and the organization of chromatin can present barriers to DNA damage recognition and repair. Epigenetic modifiers play an important role in regulating nucleosome and chromatin structure to facilitate DNA repair. For instance, epigenetic modifiers may (1) open local chromatin structure and relax nucleosome architecture to facilitate access of DNA repair proteins to damaged DNA, (2) repress transcription to prevent transcriptional machinery from interfering with DNA repair, and (3) close local chromatin structure and pack nucleosomes into a tighter conformation to restore chromatin structure back to its original state after DNA repair is completed. These roles of epigenetic modifiers in DNA repair have been described as the “prime-repair-restore” response [109]. It is hypothesized that various DNA repair pathways, including BER, MMR, SSB repair and DSB repair, can recruit epigenetic modifiers to assist in the DNA repair process during oxidative

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damage. In the literature, DSB-induced chromatin changes are well established and epigenetic changes have been studied. However, the links between epigenetic alteration and SSBs or base damage are less well studied likely due to a lack of good experimental models. Therefore, in the following sections, we will mainly focus on the roles that epigenetic modifiers play in the response to DSB repair. We will also provide a few emerging examples of the response of epigenetic modifiers to SSBs and base damage.

4.1 The role of histone ubiquitin modifiers in DNA repair

Ubiquitin is an 8.5 kDa protein that can be added to a substrate via an ubiquitin ligase to regulate the substrate's activity, function, and stability. Histone ubiquitination regulates many cellular processes, including transcription initiation and elongation, silencing, and DNA repair [110]. In response to DSBs, ubiquitination of histone 2A (H2A) functions as (1) a recruitment signal for repair proteins that contain ubiquitin-binding domains and (2) as a repressive mark that mediates transcription inhibition. For instance, after DSB induction, Ring finger protein 8 (RNF8, an E3 ubiquitin ligase) and Ubiquitin-conjugating enzyme E2 13 (UBC13) are recruited to the DSB to induce poly-ubiquitination of H2A and histone variant H2AX at lysine 63 and this modification leads to recruitment of BRCA1-A complex subunits Receptor-associated protein 80 (RAP80) and BReast CAncer genes 1 (BRCA1) to repair the DSB [111]. BRCA1 plays an important role in recruiting downstream proteins to mediate strand exchange to form primers for restoring the original sequence in homolog recombination (HR). Therefore, ubiquitination of H2A and H2AX is important for facilitating DSB repair. Recruitment of RNF8 to DSBs is dependent on its interaction with MDC1 [111], another key protein involved in DSB repair. Knockdown of RNF8 reduces poly-ubiquitination of H2AX but not mono-ubiquitination [111], suggesting mono-ubiquitination occurs through another mechanism. Recently, it has been demonstrated that in response to DSBs, another histone ubiquitin modifier, B lymphoma Mo-MLV insertion region 1 homolog (BMI-1), is rapidly recruited to sites of damage and this recruitment persists for 8 hours, resulting in mono-ubiquitination of H2A at lysine 119 [112]. Depletion of BMI-1 results in inefficient DSB repair and reduced recruitment of repair proteins including BRCA1 and RAP80 [113]. Losing either BMI-1 or RNF8 causes cells to have increased sensitivity to IR. Loss of both proteins further sensitizes cells to IR, [113], suggesting that both proteins facilitate DSB repair.

In addition to its role in promoting the recruitment of repair proteins, BMI-1 also mediates transcriptional repression in response to DNA repair. BMI-1 is one of the catalytic subunits of polycomb repressive complex 1 (PRC1). Other subunits of PRC1 include polycomb (CBX) family proteins, human polyhomeotic homolog (HPH), E3 ligase Ring finger proteins (RING), and polycomb group factor (PCGF) orthologs. Together this complex is known to mediate transcriptional repression through the ubiquitination of H2AK119 during development and differentiation [114]. Interestingly, it has recently been demonstrated that the recruitment of PRC1, including BMI-1 and RING1B, to an endonuclease-induced DSB depends on its interaction with

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phosphorylated transcription elongation factor Eleven-nineteen leukaemia (ENL), which is phosphorylated by ATM [115]. This PRC1-mediated transcriptional repression facilitates NHEJ sensor protein X-ray repair complementing defective repair in Chinese hamster cells 6 (XRCC6/KU70) to gain access to the DSB and promote repair in regions of active transcription [115].

In summary, in response to DSBs, ATM is activated and this kinase induces deposition of MDC1 adjacent to the break sites and phosphorylation of ENL. MDC1 recruits RNF8 and phosphorylated ENL recruits PRC1 member proteins including BMI-1 and RING1B to sites of damage. The recruitment of RNF8 and BMI-1 leads to poly-ubiquitination and mono-ubiquitination of H2A respectively, resulting in transcriptional repression and recruitment of downstream DNA repair proteins such as BRCA1 and XRCC6.

4.2 The role of histone methyltransferases (HMTs) in DNA repair.

HMTs are enzymes that catalyze the addition of methyl (–CH3) groups to lysine residues of histone tails and this methylation may cause transcriptional repression or activation depending on the target histone mark. For instance, di- and trimethylation of the histone residues, H3K9 and H3K27 are associated with closed chromatin structure and transcriptional repression, whereas di- and trimethylation of H3K4 are associated with open chromatin structure and transcriptional activation. Euchromatic histone-lysine N-methyltransferase (Ehmt2/G9a) catalyzes mono-methylation of H3K9, and Suppressor of variegation 3-9 homolog 1 (SUV39H1) catalyzes di- and tri-methylation of H3K9 [116]. Another lysine methyltransferase, Enhancer of zeste 2 polycomb complex 2 subunit (EZH2) catalyzes H3K27 trimethylation.

In response to DSBs, SUV39H1, G9a, and EZH2 are recruited to sites of damage where they catalyze trimethylation of H3K9 and H3K27, thereby inducing transcriptional repression in order to facilitate DNA repair. For instance, trimethylation of H3K9 occurs at DSBs through recruitment of a protein complex containing KRAB-associated protein-1 (Kap-1), Heterchromatin-1 (HP-1), and SUV39H1 (Kap-1-HP-1- SUV39H1). Deletion of SUV39H1/2 in cells causes reduction of radio-resistance and HR repair [117]. Similarly, deletion of SUV39H1/2 in mouse embryonic fibroblasts causes a mildly slower DSB repair response [118]. Importantly, the recruitment of the Kap-1-HP-1- SUV39H1 complex results in a repressive chromatin structure and activates DSB response proteins such as ATM [117]. The functional roles of Kap-1 and HP-1 in DSB repair are not clear but deletion of Kap-1 impairs the recruitment of SUV39H1 and HP-1 to DSBs and reduces H3K9me3. Recently the same group demonstrated that G9a is also involved in DSB repair. Inhibition of G9a by inhibitor BIX-0194 impairs both HR and NHEJ, resulting in an elevated radiosensitivity of glioma cells, suggesting that G9a-mediated mono-methylation of H3K9 is part of the DSB damage response [116]. DSB induction also causes an immediate but transient increase of H3K27me3 at sites of damage. The induction of H3K27me3 coincides with rapid recruitment of EZH2 and formation of γH2AX foci [119]. Deletion of EZH2 causes defective DSB repair and increased sensitivity to IR [120]. Furthermore, EZH2 also exerts its actions as part of a larger

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protein complex called polycomb repressive complex 2 (PRC2), which is known to cause transcriptional repression and gene silencing in development and cancer [121]. Other members in this complex include Embryonic ectoderm development (EED), Suppressor of zeste 12 homolog (SUZ12), Rb-associated protein 46 and 48 (RbAp46 and RbAp48). As demonstrated for EZH2, the other members of PRC2 are also recruited to sites of damage, and contribute to transcriptional repression and promotion of DSB repair [119].

The recruitment of SUV39H1 and EZH2 to chromatin is dependent on the activity of PARP-1, a DNA damage sensor protein. After DSBs occur, the binding of PARP-1 to break ends leads to recruitment of downstream DNA repair proteins such as XRCC1 and Ligase III, which are involved in alterative NHEJ repair. Additionally, binding of PARP-1 to sites of damage catalyzes the assembly of PAR chains adjacent to break sites, providing a platform for recruiting epigenetic modifiers, which compact the chromatin structure in order to block transcription and facilitate DNA repair. Specifically, PARP-1-mediated chromatin PARylation induces the recruitment of SUV39H1 and EZH2 and increase of H3K9me3 and H3K27me3 in response to DSBs [119] [117].

In summary, after DSBs occur, PARP-1 rapidly induces assembly of PAR chains adjacent to breaks sites and this modification leads to recruitment of histone methyltransferases such as SUV39H1 and EZH2. Along with SUV39H1 and EZH2 recruitment, many other proteins are also recruited to the sites of damage including G9a, Kap-1-HP-1-SUV39H1 complex members, and PRC2 members. Together these epigenetic modifiers induce repressive chromatin marks H3K9me3 and H3K27me3 to inhibit transcription and facilitate DNA repair.

4.3 The role of histone acetyl transferases (HATs) in DNA repair.

HATs are enzymes that catalyze the addition of -CH3CO groups to lysine residues within a histone tail. This addition neutralizes the charge of the lysine residue and creates an open chromatin structure that can alter gene expression. HATs are normally categorized into two different classes depending on their sequence differences and localization in different cellular compartments. Type A HATs predominately play a role in regulating chromatin-based responses in the nucleus and they contain an acetyl-binding bromodomain, whereas type B HATs, which lack a bromodomain, are normally located in the cytoplasm and only acetylate newly synthesized histones.

HAT1 is the only known example of a type B histone acetyltransferase. In response to DSB damage, HAT1 translocates to the nucleus to mediate the early stages of HR repair [122]. In yeast, Hat1p (HAT1 homolog) is recruited to chromatin in repair-defective strains (Rad52 deletion mutant) in response to DSBs [123], suggesting that HAT1 recruitment is upstream of the recruitment of DNA repair machinery. In yeast, Rad52 directs assembly of Rad51 at break ends and this promotes Rad51-

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mediated strand exchange, restoring the original sequence during HR. It was recently demonstrated that HAT1 is involved in promoting HR repair in response to DSBs in human cells via inducing marks that promote chromatin relaxation, such as H4K12Ac and H4K5Ac, around sites of damage [122]. Incorporation of these marks at sites of damage subsequently facilitates the recruitment of RAD50, a component of the MRN complex [122], suggesting that HAT1 participates in recruiting DNA damage sensing proteins. Deletion of HAT1 led to impairs HR repair efficiency, cell cycle arrest, and reduces growth rate [122].

Tat-interactive protein (TIP60) is a type A HAT that mediates acetylation of the histone proteins H2A, H3 and H4, creating an open chromatin structure. TIP60 is involved in DSB-induced DNA repair [124, 125]. After IR-induced DSBs, the MRN complex recruits TIP60 and ATM to interact with the de novo established or pre-existing H3K9me3 mark on chromatin. This binding subsequently causes enzymatic activation of TIP60, which then acetylates and activates ATM. After induction of γH2AX by ATM, activation of TIP60 also promotes acetylation of histone H3 and H4, promoting relaxation of chromatin to allow access by other repair proteins [126] [127].

TIP60 is also a subunit of the Nucleosome acetyltransferase of H4 (NuA4) complex and NuA4 is known to be involved in DSB repair. Other subunits in this complex include the cofactor Transformation/transcription domain-associated protein (TRRAP) and the ATPase p400. TRRAP plays an important role in mediating TIP60 recruitment to endonuclease-induced and IR-induced DSB sites [128]. Knockout of TRRAP causes reduced H4 acetylation and decreases recruitment of TIP60 and DNA repair proteins to sites of damage, but normal DNA damage sensing or signaling, such as activation of ATM, still occurred after induction of DSBs [128]. Interestingly, the reduced recruitment of DNA repair proteins to sites of damage in cells with TRRAP deletion is restored by relaxation of chromatin [128]. p400 is a SWI/SNF (Switching defective/sucrose nonfermenting) chromatin remodeler [129] that causes transient nucleosome repositioning to facilitate DSB repair. MDC1, which is recruited to DSBs induced by IR, is shown to physically interact with p400 and recruit p400 to DSBs. p400 recruitment facilitates nucleosome destabilization, hyperacetylation of H4 by TIP60 and recruitment of repair proteins such as BRCA1[130]. Furthermore, mutating the ATPase domain of p400 impairs the recruitment of BRCA1 to sites of damage [130].

In summary, it appears that HAT1 and the NuA4 complex (including p400, TRRAP, and TIP60) function together to relax the chromatin structure and promote recruitment of DSB repair proteins to sites of DNA damage. During this process, HAT1 is an early epigenetic modifier recruited to sites of damage and HAT1 activity recruits Rad50 to assemble the MRN complex. The MRN complex and TRRAP mediate the recruitment of TIP60 and p400, which leads to transient nucleosome repositioning to allow TIP60 to acetylate histone H4. Ultimately this process induces relaxation of chromatin and DSB repair.

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4.4 The role of histone deacetylases (HDACs) in DNA repair.

HDACs are enzymes that remove -CH3CO groups from lysine residues in histone tails, allowing chromatin to form a more closed conformation. Based on the sequence and associated cofactors, HDACs are divided into 4 classes: class I includes HDAC1, 2, 3, 8; class II includes HDAC 4, 5, 6, 7, 9; class III is a NAD^+-dependent group of deacetylases that includes Sirtuin 1-7 (SIRT1-7); class IV includes HDAC 11.

It is hypothesized that once repair starts, HDAC-mediated chromatin condensation contributes to transcriptional repression and prevents replication machinery from gaining access to DNA in order to allow the repair process to occur. Additionally, HDAC-mediated chromatin condensation terminates DNA damage response signaling [131]. It is also hypothesized that HDACs are recruited to DSB sites following HATs in order to remove acetylation marks and reset the chromatin architecture to a condensed state. In yeast, HDACs Rpd3 (HDAC1/2 homolog) and Sirtuin 2 (Sir2, SIRT1 homolog) are recruited to DSB sites after the peak of acetylation, suggesting their role is to turn off the DNA damage repair response and return chromatin structure to a closed conformation [132]. Recently, it was demonstrated in myeloma cells that HDAC8 is recruited to DSB sites to activate RAD51 to facilitate HR repair. Interestingly, HDAC8 interacts with key DNA damage response components including the MRN complex and XRCC1 [133], suggesting that HDAC8 may be involved early in the DNA damage response. Inhibition of the class I and II HDACs by trichostatin A (TSA) or knockdown of HDAC8 enhances cellular sensitivity to IR [134] [133]. This effect is possibly due to increased relaxation of chromatin caused by HDAC inhibitors, which might create an environment that renders DNA more susceptible to damage in response to IR.

In addition to contributing to DSB repair on their own, HDACs also contribute to repair as part of larger protein complexes. HDAC1 and 2 are subunits of the Mi-2/Nucleosome remodeling deacetylase (NuRD) complex. Other subunits of the NuRD complex include the Rb-associated protein 46 and 48 (RbAp46 and RbAp48), the methyl-CpG-binding domain protein (MBD2/3), the chromatin remodeler chromodomain-helicase-DNA-binding protein 3/4 (CHD3/4), and the MTA proteins (Metastasis-associated protein). During DSB repair, HDAC1 and 2 are recruited to sites of damage by MTA1 to form the catalytic core of the NuRD complex [135]. The NuRD complex is known to cause transcriptional repression and induce tumorigenesis [136]. Interestingly, PARP-1 recruits NuRD complex members MTA1 and CHD4 to DSBs to reduce nascent RNA and prevent RNA polymerase II from initiating transcriptional elongation and interfering with DNA repair following high doses of microirradiation [119]. Inhibition of HDACs by TSA promotes persistent binding of PARP-1 to DSB sites, suggesting a feedback loop exists between HDAC and PARP-1 in response to DSBs [137].

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Together, these studies suggest that following DSBs, the repair protein PARP-1 recruits MTA1 to sites of damage, MTA1 then recruits HDAC1 and HDAC2 to assemble the NuRD complex. The recruitment of the NuRD complex to DSB sites results in HDAC-dependent chromatin condensation and MTA1 and CHD4-mediated transcriptional repression.

Additionally, SIRT1 is also recruited to sites of damage where it deacetylates H4K16 during DSB repair [138]. Overexpressing SIRT1 in mice induces enhanced HR across the genome [139], whereas deletion of SIRT1 in embryos reduces genomic stability [140]. Interestingly, SIRT1 physically interacts with Tip60 and negatively regulates Tip60-mediated acetylation in response to DSBs, suggesting that SIRT1 is recruited to DNA damage sites to balance the activity of Tip60 and reset the acetylation pattern [141]. SIRT1 is a subunit within the PRC4 complex, which also includes EZH2, EED2, and SUZ12. In response to H_2O_2-induced oxidative damage, SIRT1, along with other PRC4 members, is recruited to damaged chromatin, which results in a reduction of the active transcription mark AcK16H4, an increase of the repressive transcription mark H3K27me3 and results in reduced transcription of associated genes [38]. This H_2O_2-induced SIRT1 recruitment to chromatin is dependent on the MMR proteins MSH2 and MSH6 [142].

4.5 The role of chromatin remodelers in DNA repair.

Chromatin remodelers are large protein complexes that utilize ATP hydrolysis to reorganize histone-DNA contacts, resulting in a relaxed chromatin architecture. Based on structural differences, chromatin remodelers can be categorized into four families: Switching defective/sucrose nonfermenting (SWI/SNF), Imitation SWItch (ISWI), Chromodomain-Helicase-DNA binding family (CHD) and DNA helicase INO80.

SWI/SNF, CHD, and INO80 are known to be associated with DSB repair. The mammalian SWI/SNF Brahma-Related Gene 1/Brahma (BRG-1/ Brm) is known to directly interact with chromatin to promote phosphorylation of H2AX in response to IR-induced DSBs. Knockdown of BRG-1 attenuates the formation of γH2AX, causes inefficient DNA repair and decreased cell survival in response to DNA damage [143]. A recent study showed that two acetyltransferases Nu4A and Gcn5 are required to promote the recruitment of SWI/SNF and γH2AX formation in response to DSBs likely because SWI/SNF proteins contain an acetyl-binding bromodomain. [144]. In the basal state, CHD3 interacts with SUMOylated Kap-1 in heterochromatic regions to facilitate chromatin compaction. In response to DSBs, ATM phosphorylates Kap-1 and this phosphorylation causes dissociation of CHD3 from Kap-1, thereby inducing chromatin relaxation surrounding DSB sites [145]. Inactivation of CHD3 causes aberrant heterochromatin formation and activation of Kap-1 regulated genes [145]. In response to ROS-induced base damage and DSBs, CHD4 is recruited to sites of damage and promotes further recruitment of epigenetic modifiers including DNMTs, EZH2 and G9a, resulting in aberrant DNA methylation and gene silencing. Knockdown of CHD4 causes activation of silenced tumor suppressor genes including genes important for cell proliferation, invasion and metastases in colorectal cancer

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[146]. As observed with SWI/SNF, CHD3, and CHD4, INO80 is another chromatin remodeler that is rapidly recruited to sites of damage in response to DSBs. It was demonstrated that INO80 interacts with γH2AX and is recruited to sites of damage to promote chromatin remodeling and DNA repair [147]. Mutating INO80 leads to increased sensitivity to IR-induced DSBs, suggesting that INO80 is necessary for facilitating DSB repair [148].

The ISWI family of proteins is known to be involved in both BER and DSB repair. Adding ISWI protein from yeast to a folded oligonucleosome array in vitro enhances DNA synthesis by Polymerase β (Pol β), suggesting that ISWI-mediated chromatin remodeling is important for the gap filling step of BER [149]. In regard to human ISWI, the catalytic subunit Sucrose nonfermenting-2h (SNF2H) is rapidly recruited to DSB sites. This recruitment is dependent on Sirtuin-6 (SIRT6), Ring Finger Protein 20 (RNF20), Nuclear mitotic apparatus protein (NuMA) and PARP1/3, suggesting that many upstream signals such as deacetylation, ubiquitination, and PARylation can induce recruitment of SNF2H during DSB repair (reviewed in [150]). The functional role of SNF2H in DSB repair is to promote downstream recruitment of additional repair proteins such as BRAC1 in HR [150].

4.6 The role of DNA methyltransferases (DNMTs) in DNA repair

DNA methylation is a process by which –CH3 groups are added to cytosine or adenine DNA bases by DNMTs using their substrate S-adenosyl methionine (SAM). In humans, the majority of DNA methylation occurs on cytosines that are adjacent to guanines (CpG dinucleotides) and regions of dense CpGs are called CpG islands. Methylation of promoter CpG islands plays an important role in both development and cancer. DNMT1, the most abundant DNMT, preferentially methylates hemimethylated DNA in mammalian cells, whereas the DNMT3 family (DNMT3A and 3B) methylate both hemimethylated and unmethylated CpGs.

It was hypothesized that the DNMTs may participate in restoring DNA methylation patterns on the newly synthesized DNA strand after repair. Both DNMT3B and DNMT1 are recruited to endonuclease-induced DSBs [138]. DNMT1 is also recruited to DSBs induced by laser microirradiation [151]. However, recent studies show that DNMT1 recruitment has similar kinetics to the recruitment of DNA repair proteins in response to DSBs, suggesting that transient recruitment of DNMT1 might contribute to the DNA repair process rather than being involved in restoring DNA methylation after repair [152]. After DSB damage occurs, the break ends are processed to single stranded DNA; this event is followed by loading of Replication protein A (RPA) protein and the 9-1-1 complex (Rad9-Hus1-Rad1) onto the uncovered region of the single-stranded DNA [153]. The 9-1-1 complex is a PCNA-like sliding clamp that tethers other repair proteins or checkpoint proteins to regulate DNA repair and the cell cycle. Interestingly, one of the proteins that the 9-1-1 complex (particularly Rad9) recruits and interacts with in response to DSBs is DNMT1[151], supporting the hypothesis that DNMT1 is directly involved in the DNA repair process. Further

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supporting a role for DNMT1 in DNA damage repair is the observation that HCT116 DNMT1 knockout cells exhibit enhanced sensitivity to damage including increased γH2AX and activation of DNA damage sensing proteins including ATM following laser micro-irradiation [151]. DNMT1 physically interacts with PCNA, which is known to be involved in BER and MMR, suggesting that DNMT1 recruitment to chromatin is not only induced by DSB repair but also may be involved in BER and MMR [151]. Indeed, it has been demonstrated that DNMT1 is recruited to damaged chromatin as early as 30 min after treatment of cells with H_2O_2 and this recruitment is associated with reduced transcription of genes. However, in this setting DNMT1's recruitment is dependent on MSH2 and MSH6 instead of PCNA [142].

DNMT1 acts as a scaffold protein to faciliate anchoring of other epigenetic proteins to chromatin [154]. For instance, binding of DNMT1 to sites of oxidative damage formed in GC-rich regions of the genome promotes formation and recruitment of a large epigenetic silencing complex that also contains PRC4 members and DNMT3B. Localization of these epigenetic modifiers to sites of oxidative damage in promoter CpG islands results in increased DNA methylation and reduced expression of associated tumor suppressor genes [142] [38]. Furthermore, the interaction of this large protein complex with damaged chromatin depends on MSH2 and MSH6 [142]. Interestingly, the DNA demethylase, Ten-eleven translocation 2 (TET2) interacts with DNMT1 after H_2O_2 possibly to reverse aberrant DNA methylation gains at sites of oxidative damage [155].

4.7 The role of ncRNAs in DNA repair

Although it is debatable in the field of epigenetics whether changes in expression of ncRNAs should be categorized as epigenetic alterations, it is well established that in inflammation and cancer, aberrant expression of ncRNAs are involved in regulating many genes by post-transcriptional alteration of RNA. The role of ncRNAs in DNA damage and repair is not well understood; however, studies have demonstrated that DNA damage and repair are associated with changes in miRNA (a subclass of ncRNAs) expression patterns. For instance, after IR, the expression of 34 miRNAs are altered in different types of cancer cells (reviewed in [156]). Intriguingly, one of the miRNAs that is down regulated by IR, miR-521, has a functional role in regulating DNA repair since its downstream target, a repair protein called Cockayne syndrome protein A (CSA/ ERCC8), is involved in nucleotide excision repair. IR reduces the expression of miR-521 and subsequently increases expression of CSA. Interestingly, overexpressing miR-521 enhances the sensitivity of prostate cells to IR treatment [157]. Also, in response to IR, ATM-mediated activation of p53 leads to increased expression of miR-34a/b/c. Increased expression of miR-34a/b/c is known to promote cell cycle arrest and apoptosis by reducing Cyclin dependent kinase 4 /6 (CDK4/CDK6) and BCL-2 expression [158]. p53 is also known to induce expression of other miRNAs such as miR-192 and 215, and these microRNAs have functional roles in the regulation of cell proliferation because they target the transcripts of Cell division cycle 7 (CDC7) and Cullin-5 (CUL5) [158].

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DNA damage also induces alterations in processing and maturation of miRNAs [159]. For instance, after DSB damage, ATM phosphorylates KH-type splicing regulatory protein (KSRP) and stimulates its activity leading to recruitment of primary miRNAs to Drosha for processing, resulting in increased expression of miR-16 and miR-21 [160]. Importantly, these two miRNAs have functional roles in tumorigenesis. In particular, miR-16 is involved in apoptotic signaling through regulating expression of the proapoptotic protein B-cell lymphoma 2 (BCL-2) and miR-21 is known to promote tumorigenesis by regulating cellular redox balance via targeting SOD3 and TNF-α.

Several reports have demonstrated that miRNAs can directly target ATM including miR-421, 100, 101, and 18a (reviewed in [161]). For instance, induction of miR-421 inhibits ATM expression by targeting the 3' untranslated region (UTR) of ATM transcripts, resulting in inefficient DNA repair, impaired cell cycle checkpoints and increased radiosensitivity [162]. Overall, the findings discussed above provide insight into how DNA damage alters miRNA expression patterns and also how miRNAs can in turn alter DNA damage response pathways.

It was recently shown that another group of small ncRNAs (about 21 nucleotides long) are generated from sequences that are in the vicinity of DSBs, referred to as diRNA (DSB-induced small RNAs) [163]. The role of these diRNAs at DSBs is not well understood but it appears that they participate in promoting repair. For instance, after treating cells with Ribonuclease A (RNase A), the recruitment of MDC1 to sites of damage is reduced in response to IR, suggesting that these damage site-specific diRNAs play an important role in promoting recruitment of DSB repair proteins [164].

4.8 Stable epigenetic alterations caused by recruitment of epigenetic modifiers during DNA repair.

Epigenetic modifiers can be recruited to sites of DNA damage to modify local chromatin structure and inhibit transcription during DNA repair or to restore chromatin to its original state immediately after DNA repair. However, if these epigenetic modifiers do not leave the damaged sites after DNA repair is completed; transient covalent modifications may become aberrant, stable epigenetic alterations that are heritable across cell generations. This scenario might arise if there is repetitive DNA damage during conditions such as chronic inflammation or sustained exposure to genotoxic agents, which can induce longer retention of epigenetic modifiers at sites of DNA damage. Studies have shown that sustained localization of epigenetic modifiers at certain regions of chromatin can induce stable epigenetic silencing of genes during development and cancer. For instance, during development, localization of the histone methyltransferase SUV39H1 to the *octamer-binding transcription factor 4* (*Oct4*) gene induces tri-methylation of H3K9 at the *Oct4* promoter, *Oct4* gene silencing, and reduced embryonic stem cell renewal [165]. Similarly, the histone ubiquitin modifier BMI-1 binds the promoter region of *Homeobox C13* (*HoxC13*) and induces H2A-K119 ubiquitination, resulting in gene silencing of *HoxC13* during development [166]. After viral infection, HDAC3 is recruited to *interferon-A* (*IFN-A*) promoters to reduce acetylation of H3K9 and H3K14, which are activating histone

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marks, resulting in *INF-A* gene silencing [167]. A similar persistence of epigenetic modifiers at sites of DNA damage may therefore lead to gene silencing. Currently, there is some evidence that transient recruitment of epigenetic modifiers can result in stable epigenetic alterations and gene silencing in the context of DNA repair. DNMT1 is localized to chromatin at DSBs induced by endonucleases that are repaired by HR. DNMT1 localization results in increased DNA methylation near the site of recombination and altered expression of the repaired gene [168]. DNMT3A and Ubiquitin-like, containing PHD and RING finger domains, 1 (UHRF1) and Growth arrest and DNA-damage-inducible protein GADD45 alpha (GADD45A), additional proteins that regulate DNA methylation, are also recruited to the induced DSB and participate in the alteration of DNA methylation [169]. Furthermore, inducing a DSB in the promoter region of a gene can result in recruitment of epigenetic modifiers such as DNMT1, DNMT3B, SIRT1 and EZH2 to the site of damage and persistent localization of the modifiers after repair has been completed. In a small percentage of cells the DSB causes gene silencing through repressive chromatin modifications and increased DNA methylation of the gene's promoter CpG island [138]. Recruitment of DNMT3B through SIRT1 to the site of damage is required for increases in DNA methylation in the silenced promoters. Collectively, these studies suggest that recruitment of epigenetic modifiers during DSB repair may lead to heritable epigenetic alterations and gene silencing.

A recent in vivo study demonstrates that inflammation induces the recruitment of epigenetic modifiers to sites of oxidative damage, resulting in heritable epigenetic alterations. Acute infection of mice with inflammation-inducing bacteria, ETBF, initially results in increased levels of 8-oxo-G in inflamed distal colon epithelium prior to tumorigenesis. The increased level of 8-oxo-G is associated with increased recruitment of DNMT1 and EZH2 to sites of oxidative DNA damage and increased enrichment of the histone repressive mark H3K27me3 at the promoter of candidate tumor suppressor genes, including *Hoxa5* and *Polg*. Eight weeks following infection, inflammation-induced tumors in the distal colon have regions with increased levels of DNA methylation including the promoters of *Hoxa5* and *Polg* relative to uninflamed epithelium and non-inflammation-induced tumors. Loss of the mismatch repair protein MSH2 abrogates inflammation-induced recruitment of DNMT1 and EZH2 to sites of acute oxidative DNA damage and DNA methylation alterations observed in inflammation-induced tumors [37]. These findings suggest that repair of oxidative base damage initially recruits epigenetic modifiers and sustained localization of these modifiers at sites of damage results in epigenetic alterations. Further work is needed to link SSB and other types of base damage with recruitment of epigenetic modifiers that lead to heritable epigenetic changes and permanent gene silencing.

5. Conclusion

Infectious and non-infectious agents as well as chronic disease conditions induce chronic inflammation. At sites of chronic inflammation there are increased levels of ROS that cause DNA damage and are associated with epigenetic changes, which can increase one's risk of developing cancer. The underlying mechanism for the initiation of these epigenetic alterations is unclear

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but it could be related to the role of epigenetic modifiers in DNA damage repair. ROS induces many types of DNA damage that are repaired by multiple DNA repair pathways including DSB repair, SSB repair, BER, and MMR (Figure 1). These DNA damage repair pathways recruit epigenetic modifiers such as histone ubiquitination modifiers, HMTs, HATs, HDACs, DNMTs, and ncRNA to assist in DNA repair, activate DNA damage response pathways, and regulate local transcription in response to DNA damage. Repetitive damage induced by chronic inflammation and oxidative stress may cause the transient changes induced by these epigenetic modifiers to become permanent epigenetic silencing events and give cells a selective advantage for survival and proliferation (Figure 1).

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6. Conflict of interest

The authors declare that there are no conflicts of interest.

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Figure Legends

Figure 1. The role of epigenetic modifiers in repair of DNA damage associated with chronic inflammation. Sources that can trigger the induction of inflammation include bacteria, parasites, viruses, acid reflux, inhalable particles, smoking, overconsumption of alcohol, and metabolic syndrome. Chronic inflammation can lead to accumulation of ROS which can subsequently cause oxidative DNA damage, DSBs, SSBs, single base damage and clustered base damage in surrounding epithelial cells. This damage initiates different repair pathways such as DSB repair, SSB repair, BER, and MMR by recruiting early responders such as ATM, PARP1, OGG1 and MSH2-MSH6, respectively. During repair, the following epigenetic modifiers are recruited to sites of DNA damage: chromatin remodelers, including SWI/SNF, ISWI, INO80 and CHD; histone ubiquitin modifiers, including BMI-1, RNF8; histone methyltransferases, including G9a, SUV39H1 and EZH2; histone acetyltransferase HAT1 and TIP60; histone deacetylases HDAC1, 2, 8 and SIRT1; DNA methyltransferases DNMT3B, DNMT1 and DNMT3A. In most instances, after DNA repair is completed, these epigenetic modifiers leave the site of damage, and transcription resumes. However, on rare occasions, persistent localization of these epigenetic modifiers may result in stable epigenetic changes that alter gene expression.

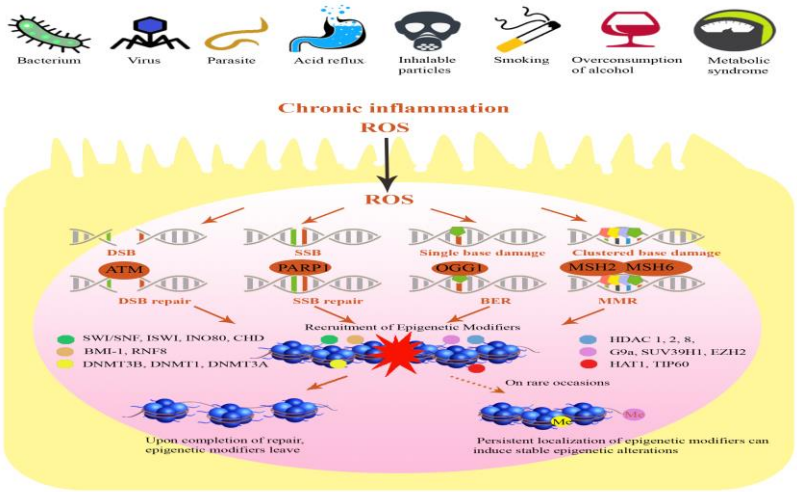

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Table 1. Types of DNA damage and epigenetic alterations associated with chronic inflammation.

Sources that can trigger the induction of inflammation		Inflammation	Types of DNA damage	DNA methylation	Histone modification	miRNA expression
Chronic inflammation caused by infectious agents	Helicobacter pylori (H. pylori)	Gastritis	↑ Base damage ↑DSBs [9]	↓ Genome-wide DNA methylation [10] [11]	↓ Global H3Ser10ph ↓ Global H3K23Ac [12]	↑ miRNA-21 ↑ miRNA 222 ↑ miRNA-223 ↓ miRNA-185 ↓ miRNA-204 ↓ miRNA106b [13]
	Schistosoma or blood-flukes	Schistosomiasis	↑ Oxidative base damage [15]	↑ DNA methylation at the promoter region of *RASSF1A* and *TIMP3* [16]	N.A.	↑ miRNA-223 ↓ miRNA-454 [17]
	Hepatitis B and C viruses	Hepatitis	↑ Oxidative base damage [20]	↑ DNA methylation at the promoter of *CDKN2A*, *CDH1*, and *IGFBP-3* ↓ DNA methylation at the promoter of *CDH6* and *ALDH1*[21]	↑ H3K4me3 at the promoter of *C-MYC* ↓ H4R3me2, ↓ H4K16Ac, ↓ H4K20me3 at the promoter of *PTEN*, *EGFR*, and *IGFBP* [22] [23]	↑ miRNA-602 ↓ miRNA-152 [24]
	Neisseria gonorrhea or Chlamydia	Pelvic inflammatory diseases	↑ DSBs [25] [26]	N.A.	↑ Global H3K9me3 [26]	↑ miRNA-155 ↑ miRNA-142-3p

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						↑ miRNA-174-3p ↑ miRNA-105 ↑ miRNA-132 [27]
	Human papillomaviruses	HPV-infection	↑ Oxidative base damage ↑ DSBs [31]	↑ DNA methylation at the promoter of *CDH1*, *INF- κ,* and *hTERT* [32]	N.A.	↓ miRNA-145 [33] [34]
	Enterotoxigenic Bacteroides fragilis (ETBF)	Colitis	oxidative DNA base damage and DSBs [36] [37]	↑ DNA methylation at the promoter CpG island of *Hoxa5*, *Polg*, and *Tnfrsf13b* [37]	↑ H3K27me3 at the promoter of *Hoxa5* and *Polg* [37]	N.A.
Sources that can trigger the induction of inflammation		Inflammation	Types of DNA damage	DNA methylation	Histone modification	miRNA expression
Chronic inflammation caused by non-infectious agents and diseases	Gastric acid, bile acid salts, and proteases	Reflux esophagitis or Barrett's esophagus	↑ DSB [39, 40]	↑ DNA methylation at the promoter region of *APC*, *TIMP3*, and *hTERT*. ↓ Genome-wide DNA methylation [41]	N.A.	↑ miRNA-21 ↑ miRNA-25 ↓ miRNA-205 ↓ miRNA-203 [42, 43].
	Asbestos fibers and silica particles	Asbestosis and silicosis	↑ Base oxidation damage [49] ↑ SSBs [49] ↑ DSBs [50]	↑ DNA methylation at the promoter of *MGMT*, *p16*, *RASSF1A*, and *PTEN* [51] [52] [53]	N.A.	↓ miRNA-181 ↓ miRNA-29 [54]
	Cigarette smoking	chronic obstructive pulmonary disease (COPD)	↑ Base damage [58] ↑ SSBs [59] ↑ DSBs [60]	↑ DNA methylation at the promoter CpG sites of tumor suppressor genes ↓ DNA methylation in Global scale [62]	↓ Global H4K16Ac ↓ Global H4K20me3 ↑ Global H3K27me3 [63]	↓ miRNA-218 ↓ miRNA-15a ↓ miRNA-199b ↓ miRNA-125b [65] [66]

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	Heavy alcohol consumption	chronic pancreatitis and liver cirrhosis	↑ Oxidative base damage [71] ↑ Acetaldehyde-DNA adduct and ↑ *N2*-ethyl-2′-deoxyguanosine [72] [73]	↓ Global DNA methylation [76]	↓ Global H3K9me2 ↓ Global H3K4me2 ↑ Global H3Ser10ph ↑ Global H3Ser28ph [77] [78]	The miRNAs that are involved in lipid metabolism, iron and glucose maintenance, and liver regeneration [79]
	Obesity and Type 2 diabetes	Systematic inflammation	↑ DSBs [84, 85] ↑ Base oxidation [83]	↓ DNA methylation at the promoter of *TNF-α* ↑ DNA methylation at the promoter of *ARNTL*, *CDKN2A*, *PPARGC1A*, and *PDX1* [86] [87]	↑ H3K9me3 ↑ H3K27me3 ↓ H3K14Ac at the promoter at metabolism-related genes [88]	↓ miRNA-143 [89]