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Chapter

Epigenetics and DNA Repair in Cancer

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Abstract

Cells can use chemical modifications in chromatin to regulate accessibility to DNA to the repair complexes and to prevent transcription in case of damage. We analyzed the relationship between repair systems and epigenetic mechanisms in DNA and RNA. We searched the PubMed database for genes involved in DNA damage response (DDR) and methylation in mRNA and DNA repair, in cancer. Epigenetic modifications, particularly histone modifications and nucleosome remodeling, trigger a signaling cascade of kinases in DNA damage response (DDR) toward efficient repair. SWI/SNF remodelers promote the recruitment of repair factors in DNA, such as DNA double-strand breaks (DSBs) that activate kinases in DDR. RNA methylation via m6A has recently attracted attention as a possible alternative pathway for repairing DNA damage. m6A is a dynamic methylation mark on mRNA that accumulates after UV irradiation and regulates transcription to facilitate DNA repair. Currently, studies seek to understand how signaling pathways activate proteins in the early response to damage. The repair maintains DNA integrity, which is a challenge in cancer because this process also represents a potential barrier to anticancer agents. The impact that epigenetic regulation can have on DNA repair is beginning to be understood.

Keywords: nucleosome remodeling, SWI/SNF complex, m6A, methylation, cancer

1. Introduction

Cells are exposed to a vast amount of exogenous genotoxic agents, such as ionizing radiation or UV light, or endogenous agents, including reactive oxygen species (ROS), derived from oxidative respiration or replication processes that can cause errors in the nucleotide chains. This damage interferes with different biological or metabolic processes, e.g., replication and transcription [1]. If these alterations are not properly repaired, then mutations, chromosomal aberrations, genomic instabilities, and other harmful effects can occur, triggering alterations such as carcinogenesis. Cells developed complex systems to deal with these problems, as they have repair systems that are activated in response to checkpoints in the cell cycle to prevent cycle progression to eliminate damage or send cells into apoptosis, when repair is no longer possible. In cancer, the response to damage is mainly activated by genotoxic agents, double-strand breaking (DSBs) repaired by homologous recombination (HR) or non-homologous end joining repair (NHEJ). Cells have signaling networks to supervise the integrity and fidelity of the major

events of the cell cycle (checkpoints) until they recognize and respond to DNA structure damage and repair. This damage response cascade is known as DNA damage response and is responsible for control of genome stability after DSBs' formation [2, 3].

Epigenetic modifications are alterations at the DNA level that do not cause permanent change in the sequence but might also cause conformational modifications. Here, chromatin plays an essential role, such that various damage response factors can gain access to the DNA sequence. Chromatin can be modified by histone changes, ATP-dependent nucleosome remodelers, and non-histone proteins, including chaperones or a high mobility group (HMG). It demonstrates that reorganization of the dynamic chromatin structure is an intrinsic component of efficient DNA repair and DDR [4–6]. Epigenetic modification has gained relevance in recent years, which involves a change in RNA. In addition, 6-methyladenosine (m6A) methylation is one of the most common RNA modifications, and is visible in eukaryotic species, such as yeast to mammals and prokaryotes and bacteria and mycoplasma. There has recently been substantial progress in m6A epitranscriptomics in its role in the initiation and progress of cancer. Studies on links between m6A and cancer yield different results in diverse tumors, suggesting that the effect of m6A modification can be variable: it affects proliferation, growth, invasion, and metastasis, but the involved pathways are just beginning to be unveiled [7, 8].

In this chapter, we will address chromatin modifications on DDR and how they function as therapeutic targets in cancer. Pathways that repair also create extraordinary work in maintaining DNA integrity, but in cancer, they are a challenge, as they represent a potential barrier to anticancer agents.

2. Chromatin dynamic in the DNA damage response

The damage response triggers the rapid recruitment of repair proteins and checkpoint activation at the site of injury in DNA structure, a crucial step for DDR signaling pathway initiation. Cancer cells are characterized by deregulation in the signaling pathways that control checkpoint homeostasis, when genes associated with DDR suffer mutations, as the injury cannot be repaired correctly and is accumulated in the genome, triggering cellular transformation [9, 10]. Signaling pathways most affected will be those of apoptosis, cell cycle, and repair, contributing to harmful effects on genome integrity, thus increasing the risk of cancer [11]. Loss of function by germlines or somatic mutations of DDR-associated genes can trigger the inability of the repair single-strand break (SSBs) or DSBs, causing cell death [12]: this is the most deleterious type of DNA damage (since a single unrepaired DSB can be lethal).

The cell develops different repair mechanisms, such as base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), NHEJ and homologous recombination (HR) as well as homologous recombination repair (HRR) [2]. The specific type of DNA repair will be activated according to the lesion, the cell cycle phase, the genomic location, and the chromatin environment [3]. However, for this to occur, the processes associated with DNA repair pathways must overcome the physical chromatin condensation barrier and packaging to gain access, detect, and repair the damage. Cooperation with different histone modifications and nucleosome remodelers are involved in DNA repair [13]. The chromatin structure functions as part of the machinery regulating genome stability and provides necessary tools to carry out basic cellular processes for genetic information integrity.

Chromatin remodelers can alter or modify the chromatin structure, catalyzing the disruption of DNA-histone contacts and displacing or evicting nucleosomes

with ATP hydrolysis to gain access to DNA. They can then regulate the stiffness, flexibility, and mobility of chromatin within the nucleus [14] or facilitate the accessibility of TFs to functional DNA elements, such as promoters or enhancers. Several ATP-dependent chromatin remodeling complexes have been directly implicated in DSB response. In yeast, INO80, SWR1, switch/sucrose non-fermenting (SWI/SNF), and remodeling the structure of chromatin (RSC) complexes are recruited to the DSB and reconfigure the nucleosomes around it so as to facilitate DNA repair and/or to modulate checkpoint activation. HMG B family is specifically involved in DSBs' repair while promoting end joining in NHEJ *in vitro* [4, 5, 15, 16].

The SWI/SNF complex regulates the correct recruitment of repair factors for NHEJ and HRR and, signaling of DDR generated by DSBs [6]. In different cancers, up to 20% of the genes, mutated or altered, belong to the SWI/SNF complex [17]. Two subunits differ according to their composition, called Brahma-related gene 1 (BRG1)-associated factor (BAF) and BRG1 polybromo-associated factor (PBAF). Further, two ATPases BRM (SMARCA2) or BRG1 (SMARCA4) are mutually exclusive but structurally related [18, 19]. On the other hand, ARID1A/1B/2, PHF10, DPF1/2/3, PBRM1 (BAF180), beta-actin, SMARCE1 (BAF57), BCL7A/B/C, BCL11A/B, SS18, and BRD9, are subunits found in mammals, so it is probable that these proteins' function are related to evolutive strategies in chromatin regulation associated with greater complexity and/or specificity to the SWI/SNF complex focalization [17]. In particular, the subunits SMARCA4, SMARCB1, ARID1A/B (BAF250A/B), PBRM1, and ARID2 have tumor suppressor function [20, 21]. this lack or silencing of a single protein, belonging to each subunit, can affect the interaction with other components of the SWI/SNF complex. The response to DNA damage was observed as nucleosome remodelers that interact through bromodomain with histone modifications and epigenetic marks (**Figure 1**).

Some functions for SWI/SNF subunits make it possible to understand the importance of this complex in cancer. Erket et al. [22] identified SMARCB1 as often lost or altered in malignant rhabdoid-type tumors. In addition, Agaimy et al. [23] and Nombiraan et al. [24] identified how alterations in SMACA4 compromise patients with non-small cell lung cancer, which clarified the prognosis, diagnosis, and personalized therapeutic potential in patients with mutated or altered SMARCA4. In the study by Yoshida et al. [25], SMARCA4 loss of function is related to thoracic sarcomas. However, Herpel et al. [26] suggest that SMARCA4 and SMARCA2 subunits, catalytic centers of SWI/SNF, should be added to diagnostic evaluation panels for lung adenocarcinomas, this is supported by results obtained in their work on protein expression by IHC, in more than 300 patients with non-small cell lung cancer. As earlier described, the proteins of the SWI/SNF complex are involved in multiple mechanisms of DDR and DSB, while NER stands out, involved with other epigenetic modifications. This is demonstrated by Lee et al. [27], who observed that Brg1 subunit interacts with acetylated H3 in addition with H2AX in early stages of DNA damage, facilitating signaling, or DDR. Particularly in DNA repair, Ribeiro et al. [28] showed that both BRM and BRG1 promote normal TFIIH (ERCC2) function in transcription and NER by regulating the expression of the GTF2H1 gene and found that cells with permanent BRM or BRG1 loss can restore GTF2H1 expression levels. Therefore, DNA damage sensitivity of BRM or BRG1 deficient cells correlates with GTF2H1 protein levels and can be used to select SWI/SNF-deficient cancers that are more sensitive to platinum drug chemotherapy. In studies carried out by Decristofaro et al. [29] and Watanabe et al. [30] in breast and lung cancer cell lines, respectively, they identified that expression of ARID1A or ARID1B was decreased or absent. They also found similar behavior in other SWI/SNF subunits (BRG1, BRM, BAF60a, BAF60c, BAF53a, and

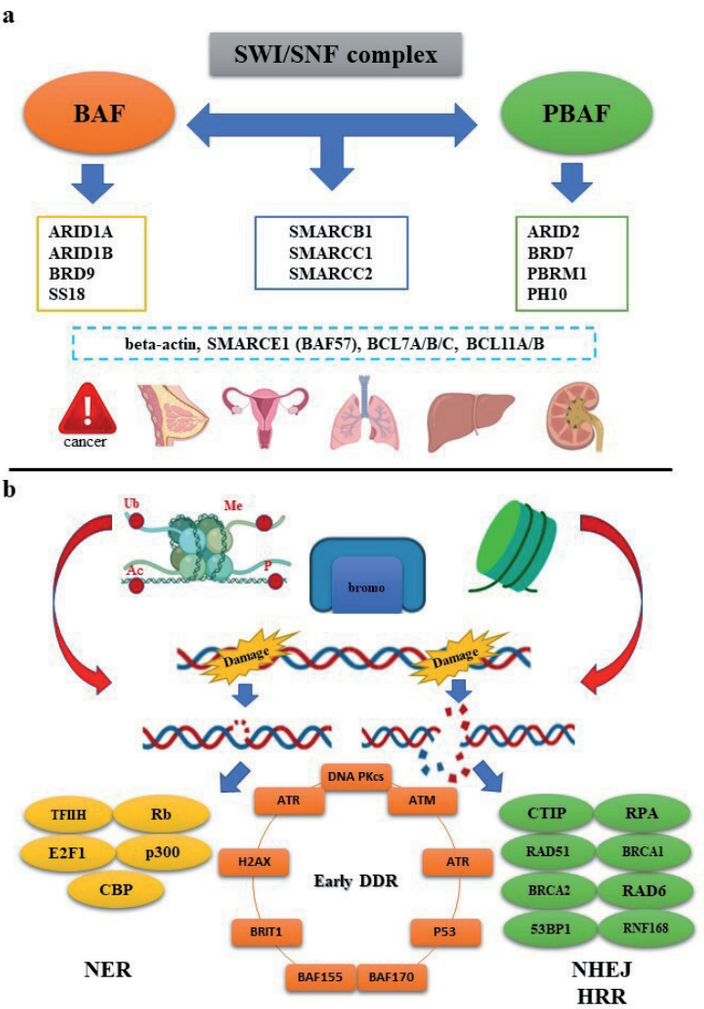

Figure 1.
The SWI/SNF complex in DNA damage response (DDR). (a) The biochemical characteristics of the BAF and PBAF subunits make the SWI/SNF complex a very heterogeneous family with exclusive properties in mammals. Alterations in this group of nucleosome remodelers give rise to cancer. (b) During DDR, SWI/SNF subunits interact through their bromodomains with histone modifications and epigenetic labels. For DNA repair to occur, it is necessary to activate the kinase cascades by ATM, responsible for recruiting and phosphorylating different repair genes such as E2F1, BRCA1 or 53BP1 and thus activating NER (nucleotide excision repair), HRR (homologous recombination repair)or NHEJ (non-homologous end joining), Ub: ubiquitination, Me: methylation, Ac: acetylation, P: phosphorylation, bromo: bromodomain.

SMARCB1). Duan et al. [31] identified that expression of ARID2 was significantly downregulated in hepatocellular carcinoma compared to adjacent nontumor tissue. Their research revealed that ARID2 inhibits cell cycle progression and tumor growth by interacting with the Rb-E2F signaling pathway. The relationship of E2F with epigenetic modifiers has become clear. Manickavinayaham et al. [32] demonstrated that the bromodomains of related acetyltransferases, p300 and CBP, specifically bind to the acetylated motif of E2F1; they found that interaction with acetylated E2F1 is critical for p300/CBP recruitment of DSBs and induction of histone acetylation at the sites of damage. They also demonstrated that in nucleosomes flanking DSBs, p300 and CBP mediate acetylation of multiple lysine residues on H3, including H3K18 and H3K56. Biswat et al. [33] and Guo et al. [34] demonstrated that E2F2 induction of histone acetylation and chromatin decondensation in response to UV radiation promote efficient NER. Lin et al. [35] shows that ATR creates induction of E2F1 in DNA damage. ATM/ATR is known to activate P53 in response to damage and plays a central role in DDR activation. ATM plays an important role in DDR and DSBs, as it regulates several pathways of cancer and epigenetic modifications.

3. Chromatin modifications in DSB repair

DSB repair can activate two repair pathways in mammalian cells: the HRR and NHEJ. First it is active throughout the cell cycle, and second it is active in S and G2 phases [36]. Usually, NHEJ is initiated by DSB recruitment of Ku70/80 or XRCC6 XRCC5 heterodimer. Ku70/80 is DBS sensor and facilitates the downstream factors recruitment, including DNA-PKcs, PAXX, XLF, XRCC4, and ligase IV. Recruitment of downstream factors helps complete DNA repair. However, HRR involves MRE11-RAD50-NBS1 (MRN) complex to activate ATM protein kinase [37]. Also, HRR can be influenced by PKcs and act in concert with the MRN complex and the recruitment of this complex to DNA DSB by hSSB1 (single-stranded binding protein), as well as the activities of 53BP1/RIF1 and BRCA1/CtIP [38]. Cells start a cascade of phosphorylation events in response to DNA damage, mediated by three phosphatidylinositol-3-related kinases: ATM, ATR, and DNA-dependent protein kinase catalytic subunit (DNA-PKcs). The kinases activate a rapid reaction of abundant sensors for DSBs to check injuries, recruit effectors, and generate a coordinated response to maintain the genome integrity [37, 39].

Different epigenetic modifications are related to these kinases, while H2AX is an important chromatin-based substrate for phosphatidylinositol-3-related kinases, when it is phosphorylated on S139, and is named γ-H2AX. This phosphorylated form serves as a checkpoint for HR and NHEJ. The foci formation of γH2A.X is the most often observed epigenetic modification triggered by DNA damage; together with the response to DSB and the SWI/SNF complex, it promotes phosphorylation of S139 in its C-terminal region through ATM in human cells. Domains of γ-H2AX are established by contact with the DSB site. In fact, the break site defines the densities and spread of γ-H2AX [40–42]. In addition, PBAF functions in the ATM pathway silence transcription in *cis* on DSB by promoting mono-ubiquitination of H2A on K119 [43] and efficient early repair on exposure to ionizing radiation (IR), mediated by DSB and NHEJ [44]. H2AX induction by UV depends on ATR, but the formation of DSB in late stages contributes to ATM activation and the increase of H2AX. DNA-PKcs, after induction of DSB, is responsible for H2AX and chromatin remodeling factor (KAP1) phosphorylation. Furthermore, DNA-PKcs is required for chromatin remodeling in early postirradiation stages and promotes the rapid recruitment of DDR initiation proteins at DSBs sites [37]. An early modulator is BRIT, a chromatin-binding protein that forms irradiation-induced nuclear foci (IRIF) and works as a proximal factor at checkpoints in DNA, controls multiple damage sensors, and early mediators to DDR. BRIT1 colocalizes with γ-H2AX, using ATM/ATR to form the BRIT1-SWI/SNF interaction through its BAF170 and BAF155 subunits, potentiating the response to damage [45, 46].

Moreover, γ-H2AX recruits MDC in the chromatin, is phosphorylated by ATM, functioning as the protein coupling site of checkpoints and repair, such as 53BP1 and the UBC13-RNF8-BRCA1 complex. The protein 53BP1 is phosphorylated by ATM and helps in the BRCA1 recruitment: together with the post-translational modifications in the damage site, they help promote other repair proteins. ATM and RNF8 facilitate the repair of DNA DSBs in the phases G1 and G2 of the cell cycle, regardless of the repair pathway used [47, 48]. Similarly, ubiquitination in the DSB regulates the repair protein BRCA1 and P53, as well as BARD1 recruitment by 53BP1 [49]. BRCA1 and 53BP1 are necessary to regulate downstream histones phosphorylation and ubiquitination to direct the repair on NHEJ and HR. Thus, BRCA1 repairs DNA damage via HR, and 53BP1 plays a crucial role in the NHEJ repair pathway, ensuring DNA DSBs are repaired correctly [50, 51]. BRIT1 is required for DNA damage-induced intra-S and G2/M checkpoints, as regulation of the BRCA1 and Chk1 expression [52]. The loss of function of either of these two genes can alter

the HRR pathway, resulting in genetic instability and an increased risk of breast or ovarian cancer in BRCA1/2 germline mutation carriers [53, 54]. Cruz et al. [55] analyzed the RAD51 foci in 20 samples from patients with breast cancer, 10 in germinal-BRCA1, and 10 in germinal-BRCA2. The results provide new evidence of HRR restoration functionality as a frequent mechanism of PARPi resistance and demonstrate the potential of functional biomarkers to discriminate against tumors that will fail PARPi monotherapy. This emphasizes the major role played by ATM and DBSs-associated repair pathways in breast cancer treatment, whether mediated by IR or chemotherapy.

Qi et al. [56, 57] found that BRG1 decreases the nucleosomes stability at DSBs and creates an open and relaxed chromatin structure in SW13 and U2OS cells. It shows that BRG1 is crucial in early damage repair by remodeling the chromatin structure near DNA damage sites. They show that the BRG1 domain facilitates the RPA replacement with RAD51 at the DSB site in the HR, interacting with the RAD52 mediator and regulating its recruitment into the DSBs. de Castro et al. [58] demonstrated for the first time that Arid2 expression is important for HR. They found that Baf200 and Brg1 are required for efficient recruitment of Rad51 to a subset of DSBs, repaired by HR, where Rad51 and Arid2 are part of the same complex. Haokip et al. [59] demonstrated that SMARCAL1 and BRG1 regulate each other in HeLa cells with DNA damage-inducing agent doxorubicin, resulting in an increase in the SMARCAL1 transcription and protein. They found that BRG1 is present at the Enh1 and Enh2 region of SMARCAL1 promoter in untreated HeLa cells. Experiments showed how BRG1 is present on SMARCAL1 promoter, with protein occupancy increasing when DNA is damaged, indicating that BRG1 can positively regulate SMARCAL1, creating a regulatory loop. Regarding the relationship with proteins of initial DDR pathway, Keka et al. [60] showed that the loss of Smarcal1 reduces the XRCC4 recruitment to DSB sites several times and suggests that Smarcal1 is required for DNA-PKcs/Ku70/Ku80 complex to the correct functions. Diplas et al. [61] found that loss of SMARCAL1 in glioblastoma cells can induce alternative lengthening of telomere (ALT) phenotypes, in the same way as ATRX. ATRX mutations are the most prevalent abnormality in glioma, as Han et al. [62] identified that knockout ATRX inhibited glioma cell growth, tumor invasion, and a decrease in H3K9me3 availability, which can inhibit the ATM acetylation resulting in increased glioma cell chemosensitivity. They found that ATRX is involved in DNA damage repair by regulating the ATM pathway, suggesting a good prognostic marker in predicting temozolomide (TMZ) chemosensitivity.

Although little is known about the role of H1.2 in response to damage and interaction with the repair machinery proteins, its function has become clearer. Kim et al. [63] reported that p300-mediated acetylation of p53 and DNA-PK-mediated phosphorylation of H1.2 alter the p53-H1.2 interaction, thus alleviating the repressive effects of H1.2 on the transactivation of p53. After DNA damage, p53 and H1.2 undergo modifications in an orderly fashion, with H1.2 phosphorylation at T146 followed by p53 acetylation. p53-H1.2 interaction is essential to enhance p53 function, and point mutations that mimic its constitutive modifications induce efficient growth inhibition and apoptosis. Li et al. [64] described a new mechanism to H1.2, without other H1 isoforms, to regulate the DNA damage response and repair through repression of ATM recruitment and activation. Moreover, H1.2 functions as a molecular brake for ATM binding to MRN, whereas DNA damage-induced ATM activation requires both the MRN complex assembly and H1.2 release. As such, these authors revealed a new link between chromatin disturbances, destabilization of H1.2, and ATM activation. For the first time, it was found that the RNF168/RAD6 complex can promote mono-ubiquitination of histone H1.2 *in vitro*, and the H1.2 mono-ubiquitination can be induced after IR treatment. They concluded that

H1.2 mono-ubiquitination *in vitro* and *in vivo* by RNF168/RAD6 is evidence that mono-ubiquitination can establish a suitable microenvironment for other E2/E3 complexes to catalyze polyubiquitination or multi-mono-ubiquitination of H2A and H2AX over H2A and H2AX ubiquitination, which is dependent on RNF8/Ubc13 [65]. Thorslund et al. [66] proposed that linker H1 represents a key chromatin-associated RNF8 substrate, whose UBC13-dependent K63-bound ubiquitylation at DSB-containing chromatin provides a scaffold to RNF168 binding through its UDM1 module. RNF168 ubiquitinates H2A into K13/K15 and possibly other proteins to trigger repair factor recruitment in DSB. Giné et al. [67] implicated H1.2 as a valuable protein for apoptosis induction in chronic lymphocytic leukemia cells, and its release pattern is correlated with deletions of 17p and treatment response; they emphasized that histone H1.2 could be an essential apoptotic signal induced by agents acting independently of p53.

4. Methylation in DNA damage and repair

DNA methylation is considered a post-replication modification by the methyl group (–CH3) addition at carbon 5 cytosine, known as 5-methylcytosine (5mC), primarily in dinucleotides of CpG [68]. This enzymatic reaction is catalyzed by three DNA methyltransferases (DNMTs) (DNMT1, DNMT3A, and DNMT3B). These modifications are commonly found in promoter regions, the CpG islands, as their main function is transcription silencing, decreasing, or repressing the gene function. Methylation is the epigenetic modification most studied in cancer, as it is well-known that DNA hypermethylation can transcriptionally silence tumor suppressors and DNA repair genes, giving neoplastic cells survival advantages [69]. Various genes related to cell cycle regulation, tumor cell invasion, cell signaling, apoptosis, and chromatin remodeling are hypermethylated and silenced in almost all tumors. As demonstrated by Pal et al. [70] for the first time, CpG sites in the promoter of H2AX, RNF8, and CYCS are methylated; they show the collaborative participation of hypermethylation of DR5, DCR1, DCR2, CASP8, CYCS, BRCA1, BRCA2 and H2AX and the hypomethylation of DR4, FLIP, and RNF8 in sporadic breast cancer; the authors proposed that promoter methylation of these apoptotic and DDR genes is not due to a random phenomenon, as the progressive modification of aberrant epigenetic alterations are associated with tumor advancement, which generates the dysregulation of the DDR-apoptotic pathway, promoting tumor development. Hinrichsen et al. [71] found that increased methylation in the promoters of MLH1, MSH2, PMS2, and p16 genes are correlated to an advanced stage in hepatocellular carcinoma. Epigenetic marks and DDR are crucial points to understand if changes in DNA methylation can contribute to resistance of cancer treatment, particularly by radiotherapy [72].

Various epigenetic marks have been associated with the response to damage, primarily the methylation of lysine 4 and 79 in H3 (H3K4me and H3K79me, respectively) that contribute to DDR and DNA repair [73]. The marks on H3K4me1/2/3 regulate the repair of DSBs through chromatin accessibility [74, 75]. Furthermore, H3K36 methylation is associated with "open" euchromatin and helps RNA pol II activate transcription [76]. Chang et al. [77] demonstrated that the tumor suppressor PHRF1 can move on the DSB due to H3K36me2/me3 and NBS1, then ubiquitinate PARP1, and trigger the subsequent repair by NHEJ. Yet, it is known that DOT1L methyltransferase catalyzes the H3K79 mono-di-trimethylation through its non-SET domain. Recently, in the study by Kari et al. [78], the depletion or inhibition of DOT1L activity was shown to result in altered DNA damage response, indicated by decreased levels of γH2AX, but with increased KAP1 phosphorylation. Loss

of DOT1L function leads to faulty HRR-mediated DSB repair without affecting NHEJ. Highlighting DOT1L-mediated H3K79me3 importance in the early response to DNA damage and DSB repair, its inhibition also increases radiation sensitivity and chemotherapeutic agents in colorectal cancer patients' treatment. Dot1L and H3K79 methylation was previously associated with the role of 53Bp1 in response to DNA damage [79]. FitzGerald et al. [80] corroborated that not only H3K79me recruits 53BP1 to DNA damage sites, but also H4K20me. This indicates that H4K20me concentration is essential for the repair pathway related to 53BP1 and BRCA1, in cooperation with the 53BP1-RIF1-MAD2L2 complex. The histone post-translational modification of H3K27me3 and H4K20me is a diagnostic indicator in melanoma [81].

There is DNA methyltransferase, in which expression is regulated by epigenetic modification during DNA damage. MGMT is an O^6-methylguanine-DNA methyltransferase responsible for the repair of damaged guanine, without other cofactors transferring the methyl at 06-meG to cysteine residues, removing adducts in a single step, thus protecting chromosomes from mutations, carcinogenesis, and alkylating agents [82, 83]. MGMT can do this only once, after its DNA-binding domain change by alkylating, which is detached from DNA and targeted for degradation by ubiquitination [84]. Loss of MGMT expression is due to promoter methylation [85], used as an advantage for good prognosis in a glioma, which can predict whether alkylating agents can benefit treatment [86]. MGMT can interfere with TMZ response on tumor cells and is responsible for efficient repair of TMZ and induced toxic DNA adducts, reducing treatment efficacy. Targeting MGMT seems to overcome chemoresistance in gliomas, but the prognostic value of MGMT methylation is controversial, as genomic rearrangements result in MGMT overexpression, independent of its promoter methylation, contributing to resistance [87].

5. Genome damage repair via m6A participation

Modified nucleotides on DNA create a vast list, shown by Fragou et al. [88], but methylation by methyltransferases is an epigenetic modification that occurs on DNA and RNA. In fact, RNA contains more modified bases than DNA, as these RNA modifications are present in rRNA, tRNA, snRNA, miRNA, or mRNA, giving rise to the emergence of a wide chemical diversity on its side chains. One of these modifications on mRNA has been studied since its discovery in 1974 [89, 90], the methylation of adenosine residues form N6-methyladenosine (m6A) is the most abundant post-transcriptional mRNA modification, and is detected in approximately 25% of mRNAs [91]. The m6A modification mechanism of action functions like methylation on DNA and histones, i.e., its presence or absence on mRNA determines the fate of the transcription. The addition of a methyl group to the N6 site of A, occurring in the RRACH sequence [92, 93], has a distinctive position in the vicinity of stop codons, internal exons, in 5'UTRs which could promote 5'cap-independent translation when found on the first nucleotide adjacent to the 7-methylguanosine cap; this gives protection from decapping [7, 94, 95] and in 3'UTRs regulates the affinity of RNA binding proteins [96]. This is known as m6A modification, which is involved in a variety of cellular processes including gene expression through regulating RNA metabolism, such as mRNA translation, degradation, alternative splicing, export and folding [97, 98], control of protein translation [94], and others. All these effects are globally known as RNA epitranscriptomics.

For some time, m6A modification was considered static and unalterable, but is now well-known as reversible and dynamic. It is difficult to examine gene regulation at the RNA level without appropriate methods; genome-wide sequencing became a

major tool for many years, but had limitations with the m6A assay. The sensibility of the methodologies used was a critical point, because until recently, techniques had a detection limit of about 0.01%, in contrast to the content of m6A, less than 0.001% is sufficient to regulate biological processes [99]. Antibody-based, high-throughput sequencing technology allowed us for the past few years to locate the specific m6A sites and explore their biological significance. Linder et al. [91] solved the problem of distinguishing between m6A and adenosine by using incubation of an m6A-antibody to induce a specific mutational signature of m6A residues after UV light-induced antibody-RNA crosslinking and reverse transcription.

This modification is added and eliminated by proteins called "writers" and "erasers," respectively; these proteins regulate the abundance, prevalence, and distribution of m6A; this exerts its biological function and is modulated by protein "readers." First, m6A is added to the nucleus by the methyltransferase complex, which is formed by two core proteins, METTL3 and METTL4 [92]. METTL3/METTL4 form a functional heterodimer, METTL3, which is the catalytic subunit which uses S-adenosylmethionine (SAM) or S-adenosylhomocysteine (SAH) as donors for methyl transfer in adenosine within the consensus motif G(G/A) ACU [100]. METTL4 has the active site blocked, and lacks amino acid residues to form hydrogen bonds with the ribose hydroxyls of SAM to facilitate donor and acceptor substrate binding; this is important in maintaining the integrity of the complex, stabilizing METTL3, and enabling RNA substrate recognition [101]. Regarding m6A demethylases or "eraser" proteins, FTO and ALKBH5 are two well-known m6A demethylases located in nuclear speckles within the methyltransferase complex. They belong to the group of Fe(II)/2-oxoglutarate (2OG)-dependent dioxygenases [102] and act on both DNA and RNA. FTO demethylates m6A through oxidative reactions that generate two intermediate hydroxymethyl6A and formyl m6A [103]. ALKBH5 catalyzes the direct removal of m6A, oxidizing the N-methyl group of the m6A site to a hydroxymethyl group, as its m6A demethylation function affects total RNA synthesis and mRNA export [104]. The "reader" proteins belong to the YTH domain family proteins, and in humans include YTHDF1–3 and YTHDC1–2. YTHDF1 stimulates mRNA translation to interact with eIFs and ribosomes. YTHDF2 binds to m6A, located in the 3'UTR leading mRNA to become processing bodies for degradation in a methylation-dependent manner [105]. While YTHDC1 protein binds to m6A, as well as pre-mRNA splicing factor SRSF3 to its mRNA-binding elements, they are close to m6A sites but block SRSF10 mRNA binding, promoting exon exclusion, and modulating mRNA splicing by recruiting pre-mRNA splicing factors [106].

Damage to DNA is derived from replication stress, telomere shortening, UV light, chemical toxins, and ROS - but RNA can suffer alterations and respond to damage agents. This variety of DNA lesions is removed in cells by protein complexes in specific repair systems (**Figure 2**). Until recently, response to damage was unique to DNA, while analyzing the response of DNA to UV, with Xiang et al. [107] changing that view. They found an accumulation of m6A on poly(A) + RNA two minutes after UV irradiation in response to DNA damage. They observed that the methyltransferase complex (METTL3, METTL4, WTAP) and Poly(ADP-ribose) polymerase (PARP) is localized to sites of UV-induced damage, with FTO demethylase recruiting. DNA polymerase translesion (Pol κ) is necessary for METTL3 and METTL4 recruitment. The authors suggested that PARP, METTL3, m6A RNA, and Pol κ could be alternative repair pathways to respond to UV-induced damage, with m6A in the main role for rapid recruitment of Pol κ to damaged sites. Colocalization of Pol κ with m6A to sites with a high content of cyclobutene pyrimidine dimers (CPDs) corroborate that m6A RNAs have a regulatory role in the NER pathway. Svobodová et al. [108] found that m6A RNAs are diffuse to damaged DNA, but

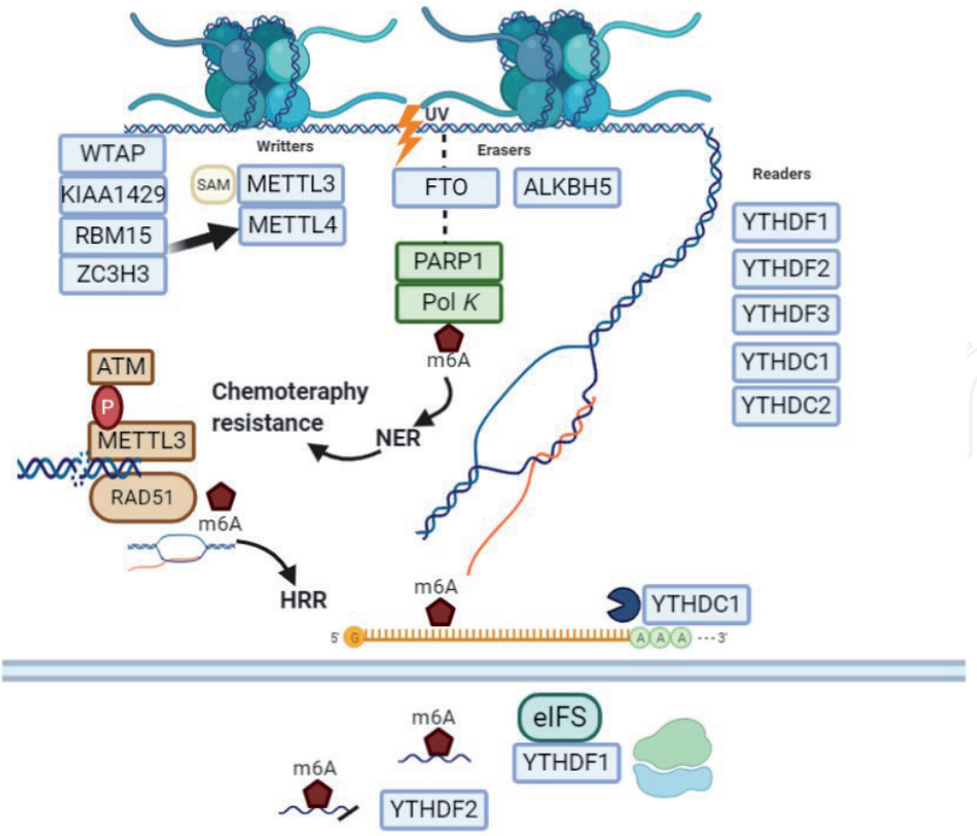

Figure 2.
Regulatory complexes of m6A in DNA damage repair. The m6A mark on mRNAs is involved in some repair pathways in response to DNA damage. It has been observed that the interaction of writing, eraser and reader proteins with other proteins that are part of canonical repair pathways is essential for the response to damage. SAM: S-adenosylmethionine; UV: ultraviolet light, NER: nucleotide excision repair, HRR: homologous recombination repair, eIFS: eukaryotic initiation factors, phosphate group: red circle.

a new participant, METTL16, accumulated 20–30 min after induced damage in a subset of irradiated cells. This response was specific to CPDs, as the authors observed that m6A RNAs' accumulation pattern was specific to repair of CPDs' sites, which do not accumulate in other lesions, such as NHEJ.

Apyrimidinic or apuric (AP) sites can arise by spontaneous hydrolysis, cleaving the N-glycosidic bond through elimination of an incorrect or damaged base by DNA glycosylases or ionizing radiation. Considered one of the most predominant lesions in the genome, it is repaired by the BER pathway, which has PARP-1 as the first sensor and responder, activated by poly(ADP-ribose) polymerase [1]. Recently, Xiang et al. [107] found it was associated with m6A in RNA. AP lyases cleave 5′ or 3′ to AP sites to further processing by DNA polymerase and ligase. ALKBH1 is another member of the AlkB family that participates in demethylation of histone H2A in mouse stem cells. It is capable of cleavage to DNA at AP sites, using a lyase mechanism to produce a DNA nick on the 3′ side of a basic site, leaving a product that is missing a 3′-phosphate and an adduct in the 5'-DNA product. ALKBH1 acts on both ss-DNA and ds-DNA and can produce DSBs related to AP lyase activity. Human ALKBH1 possesses m6A demethylation activity, although this is not its main function. It cannot be ruled out this has a role in epigenetic gene silencing. Due to its abundance in mitochondria, it could play a primary role in mitochondrial DNA repair and function [109–111]. Other AlkB, such as ALKBH3, function as RNA repair enzyme; it is equally distributed in cytosol, the nucleus, and is active on RNA and ssDNA [112]. Zhang et al. [113] showed how METTL3 is phosphorylated by ATM protein for its localization to DSBs, where it catalyzes m6A in RNAs. METTL3 stimulates the recruitment of RAD51 to DSBs in a DNA-RNA

hybrid-dependent manner, promoting efficient homologous recombination-mediated DSB repair. The upregulation of METTL3 may contribute to resistance to chemo- and radiotherapy.

6. Cancer drugs: resistance and epigenetics

Alterations in m6A and proteins responsible for its regulation on the RNA level were shown to interfere with the response to cancer treatment. Cyclophosphamide (CTX) is an alkylating chemotherapy drug used in cancer treatment; it forms adducts at the N7-guanine position, which are unstable and therefore undergo spontaneous dissociation. These features are exploited in rapidly dividing cells, which are likely to be disrupted before repair takes place [114]. Little is known about CTX effect on RNA epigenetic complexes, yet it was shown to increase m6A levels, inhibiting the gene and protein expression of FTO, YTHDF1, YTHDF2, YTHDC1, and YTHDF3 in a time- and concentration-dependent manner [115].

Cervical squamous cell carcinoma (CSCC) is an example of increased chemotherapy resistance; Zhou and colleagues [116] found that β-catenin is an FTO target: they observed FTO overexpression and reduced m6A β-catenin levels, with the effect of this change upregulation of β-catenin protein and the subsequent activation of ERCC1, a critical player in NER, which contributes to chemotherapy resistance and a poor prognosis. In case of BRCA-mutated epithelial ovarian cancers (EOC), the use of Olaparib has been clinically beneficial: it is a poly(ADP-ribose) polymerase inhibitor (PARPi), which detects and binds DNA SSBs and DSBs, using the N-terminal DNA binding domain [117, 118]. When DNA is damaged, PARP-1 can recognize damaged sites and their formation; the binding exposes the enzymatic site of PARP-1, resulting in its activation and the recruitment of XRCC1, the first protein for assembly and activation of DNA bases excision repair machinery [119]. The resistance of tumor cells to PARPi is not well-known, but in the study of Fukumoto and colleagues [120], FZD10 was observed as a receptor in Wnt/β-catenin signaling, increasing m6A modification of mRNA in resistant cells, thus stabilizing. This increase contributes to PARPi resistance by upregulating the Wnt/β-catenin pathway in BRCA-deficient EOC cells.

Xiang et al. [107] showed that FTO could be recruited to damaged γH2AX chromatin after irradiation, strengthening FTO participation in DNA damage repair. In melanoma, FTO is upregulated and promotes cell proliferation, cell migration, invasion, and cell viability. Yang et al. [121] demonstrated that FTO regulates PD-1 expression that also promotes mTOR signaling. FTO can promote resistance to anti PD-1 blockade in melanoma through m6A, mediating PD-1 (PD-1 or PDCD1 is a negative regulator of T-cell activity). This supports the anti PD-1 blockade with an anti-tumor response in advanced cancers and reduces immune-related adverse side effects, vs. with ipilimumab.

Glioblastoma is a common and aggressive primary brain tumor in adults, and is highly resistant to treatment such as surgery, irradiation, and adjuvant TMZ chemotherapy, which failed to improve the outcome. One cause of poor response to TMZ, as suggested by Visvanathan et al. [8], is that GSCs show high levels of m6A and METTL3, supporting the proposal that METTL3 is key in GSC maintenance, making those cells resistant to therapy and refractory to radiotherapy by efficient repair of DNA. Here, METTL3 alters the DNA repair efficiency and radiation sensitivity through m6A sites in SOX2–3'UTR, stabilizing it in GSC, as the recruitment of human antigen R (HuR) to m6A modified mRNA is crucial for SOX2 stabilization by METTL3. This supports a role for METTL3, shared with SOX2 in another repair pathway, mediated HR: SOX2 protects GSCs from radiation-induced cytotoxicity

by promoting HR repair, implying an oncogenic role for METTL3 and m6A. At the same time, FTO becomes a promising target to develop FTO inhibitors like Rhein, Meclofenamic acid (MA2), or its ethyl ester form, MA2 [122].

Hepatocarcinogenesis is correlated with abnormal m6A modifications, high METTL3 and YTHDF1 expression in hepatocellular carcinoma (HCC) is associated with poor prognosis, with its combination as a malignant marker, according to Zhou et al. [123]. The participation of the machinery regulating m6A was clarified by the work of Chen et al. [124]. They found a significant increase in mRNA m6A levels, supporting the role of m6A in liver cancers. When METTL3 is downregulated, it is unable to act on tumor suppressor SOCS2, being silenced by METTL3 through m6A-YTHDF2. SOCS2 transcripts are a direct target of YTHDF2 with mediated mRNA decay, promoting tumor progression. Recently, Lin et al. [125] found that METTL3 depletion leads to a resistant phenotype in HCC with sorafenib treatment through regulation of FOXO3 expression. FOXO3 m6A methylation maintains stability, but its absence accelerates degradation. The modification is read by YTHDF1, which stabilizes m6A-labeled RNA and promotes protein synthesis. These results contrast with those obtained by Taketo and colleagues [126] when they established a METTL3-KD using a pancreatic adenocarcinoma cell line. The authors concluded that these cells had higher sensitivity to gemcitabine, 5-fluorouracil, cisplatin, and irradiation. It is clear that METTL3 plays a key role in resistance to therapy, but the way this gene behaves in different types of cancer is not yet understood. In a study on colorectal cancer (CRC), it was found that c-Myc activates the YTHDF1 gene expression. YTHDF1 is overexpressed in CRC and has been associated with lymph node metastasis and poor prognosis, as the evidence in vitro with YTHDF1 knockdown indicates that cancer cells are sensitized to the exposure of 5-Fluorouracil and L-OHP (oxaliplatin) [127].

7. Conclusions

The SWI/SNF complex has been shown to be a central regulator in DNA repair, over other epigenetic complexes such as Polycomb repressive complex 1 (PRC1) and 2 (PRC2), which require the cooperation of their subunits and epigenetic markers to trigger the on–off signaling cascade, generated by acetylation, methylation, or ubiquitination of genes involved in response to DNA damage. We see that participation of epigenetics in the cellular responses goes far beyond DNA promoter methylation and histone modification. Methylation of RNA has a critical role in cell maintenance, changing our notion about RNA functions. We do not know whether deregulation of the m6A machinery could result in cancer development or progression by altering DNA damage response, but knowledge of molecular mechanisms of regulation of m6A cellular modification in tumor cells may develop a combined therapy for m6A regulator proteins as targets that facilitate a better cancer response.

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Conflict of interest

The authors declare no conflict of interest.

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