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MINI REVIEW
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The Methylation Game: Epigenetic and Epitranscriptomic Dynamics of 5-Methylcytosine

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DNA and RNA methylation dynamics have been linked to a variety of cellular processes such as development, differentiation, and the maintenance of genome integrity. The correct deposition and removal of methylated cytosine and its oxidized analogues is pivotal for cellular homeostasis, rapid responses to exogenous stimuli, and regulated gene expression. Uncoordinated expression of DNA/RNA methyltransferases and demethylase enzymes has been linked to genome instability and consequently to cancer progression. Furthermore, accumulating evidence indicates that post-transcriptional DNA/RNA modifications are important features in DNA/RNA function, regulating the timely recruitment of modification-specific reader proteins. Understanding the biological processes that lead to tumorigenesis or somatic reprogramming has attracted a lot of attention from the scientific community. This work has revealed extensive crosstalk between epigenetic and epitranscriptomic pathways, adding a new layer of complexity to our understanding of cellular programming and responses to environmental cues. One of the key modifications, m^5C, has been identified as a contributor to regulation of the DNA damage response (DDR). However, the various mechanisms of dynamic m^5C deposition and removal, and the role m^5C plays within the cell, remains to be fully understood.

Keywords: DNA, RNA, m^5C, hm^5C, methyltransferases, demethylases, cancer, DNA damage

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INTRODUCTION

Gene expression regulation is not only affected by mutations in the genomic sequence, but also by various other molecular mechanisms (Allis and Jenuwein, 2016), such as epigenetic regulation. Epigenetic regulation can influence gene expression profiles regardless of the genomic sequence, and can result in either the condensation or relaxation of the chromatin structure (Dai et al., 2020). The main players in epigenetic control; m^5C DNA methylation, histone modifications, and non-coding RNAs (Kumar et al., 2018), have been linked to genome stability and are known to contribute to the maintenance of genome integrity (Deem et al., 2012). Three distinct aspects govern epigenetic status; inheritance, environmental factors, and stability over time (Bonasio et al., 2010). Epigenetic modifications are stable enough to maintain cellular homeostasis, but are also reversible to allow transitions among different states and responses to environmental cues (Zhang et al., 2019). The dynamic nature of epigenetic features controls several aspects of transcription regulation and consequently influences many physiological processes, such as development (John and Rougeulle, 2018), transposon control (Misiak et al., 2019), and brain and memory formation (Kim and Kaang, 2017). Furthermore, deregulation of epigenetic processes can lead to genomic instability, promoting the onset and development of different diseases including cancer (Lu et al.,

2020) (Liu et al., 2017). For example, during cancer development, genome-wide DNA hypomethylation and gene-specific hypermethylation occur as a consequence of mutated or deregulated chromatin modifiers (Ibrahim et al., 2022). Thus, epigenetic alterations or epimutations (McCarrey et al., 2016) result in abnormal transcriptional repression or activation of genes (Lee, 2019). Epimutations can be classified into two groups: primary, which are epigenetic alterations in the absence of a genetic change, and secondary epimutations, which are acquired as a consequence of DNA mutations in genes of *cis* or *trans*-acting chromatin factors (Oey and Whitelaw, 2014). Interestingly, secondary epimutations are the most reported in cancers (Ruiz de la Cruz et al., 2021). At a molecular level, epigenetics involves a complex and dynamically reversible set of structural modifications of chromatin catalysed by enzymes often referred to as "writers," which add different chemical modifications such as methyl group moieties to DNA (Biswas and Rao, 2018). These molecular decorations are then able to recruit a plethora of proteins called "readers" that specifically recognise these moieties (Du et al., 2015). Finally, a set of "erasers" catalyses the removal of the deposited modification. Writers, readers, and erasers function dynamically to regulate the epigenetic landscape. Concerted variations of epigenetic modifications ensure an organism's normal development and its responsiveness to environmental stimuli (Norouzitallab et al., 2019). Removal and restoration of methylation marks is also important during embryonic development, with coordinated waves of demethylation and *de novo* methylation establishing specific cell fates (Wu and Zhang, 2014). With recent advances in high-throughput sequencing techniques and transcriptome-wide studies, more than 150 post-transcriptional modifications have been also described on RNA, termed the epitranscriptome (Ma et al., 2022). Similarly to DNA methylation, RNA bases can be methylated and can function in the fine-tuning of gene expression (Kumar and Mohapatra, 2021) (Seo and Kleiner, 2021) (Willbanks et al., 2021). Epitranscriptomic studies have also revealed how post-transcriptional RNA modifications can dynamically affect several aspects of RNA metabolism including processing, export, translation, and RNA stability (Flamand and Meyer, 2019; Ranjan and Leidel, 2019; Trixl and Lusser, 2019; Boo and Kim, 2020; Chen et al., 2021a; Kumar and Mohapatra, 2021; Schaefer, 2021). Furthermore, epitranscriptomic changes have been demonstrated to play a crucial role in stress response processes (such as the DNA damage response) (Jimeno et al., 2021; Wilkinson et al., 2021) and aberrant epitranscriptomes are associated with several human diseases, including cancer (Hsu et al., 2017; Jiang et al., 2017; Lian et al., 2018; Esteve-Puig et al., 2020). Unlike epigenetic DNA modifications, RNA methylation cannot be transferred into offspring and can result in significant changes in RNA secondary structure (Schaefer et al., 2017). Changes in base-pairing potential and protein-RNA interactions make epitranscriptomics a complex cellular mechanism that impacts both RNA metabolism and gene expression (Kan et al., 2022). Epigenetic and epitranscriptomic marks have the ability to expand the physicochemical features of the A-T-C-G nucleobases. The m^{5}C DNA modification, known as the fifth base, has been extensively described as a CpG-specific modification able to modulate chromatin architecture with the assistance of repressive histone mark deposition. More recently, significant technical progress for RNA m^{5}C detection approaches has been made (Yuan et al., 2019). Novel techniques, including m^{5}C RNA immunoprecipitation sequencing (m^{5}C-RIP-seq), 5-AZA-cytidine-mediated RNA immunoprecipitation sequencing (Aza-IP-seq) (Guo et al., 2021), methylation-individual nucleotide resolution crosslinking immunoprecipitation sequencing (miCLIP-seq) (Chen et al., 2021a) and TET-assisted peroxotungstate oxidation sequencing (TAWO-seq) (Yuan et al., 2019), were successfully developed and applied. The presence of m^{5}C has been detected in diverse RNA molecules including tRNAs, rRNAs, mRNAs, viral RNAs, and ncRNAs (George et al., 2017; Genenncher et al., 2018; Zeng et al., 2018; García-Vílchez et al., 2019; Trixl and Lusser, 2019; Zhao et al., 2020). Although the m^{5}C modification on RNA has been demonstrated to play a role in the pathogenesis of several diseases, the mechanism of action remains largely unexplored. Furthermore, evidence of changes in the transcriptional landscape driven by m^{5}C during several physiopathological processes, ranging from pluripotency, development, differentiation, genome instability, and oncogenesis have been reported (Frye et al., 2018; Bohnsack et al., 2019; He et al., 2020; Song et al., 2022). A large amount of scientific literature describes cancer as a genetic, epigenetic, and epitranscriptomic disease (Esteller and Pandolfi, 2017; Lobo et al., 2018; Porcellini et al., 2018; Xue et al., 2020a; Esteve-Puig et al., 2020; Xie et al., 2020; Miano et al., 2021; Lopez et al., 2022). Oncogenesis driven by genome instability and consequently accumulation of mutations is a complex pathological process that involves changes in gene expression. Both hypermethylation and hypomethylation at different genetic loci and of RNAs is strongly correlated to tumour initiation, progression, and metastasis (Pérez et al., 2018; Locke et al., 2019; Nishiyama and Nakanishi, 2021). Furthermore, deregulated deposition and removal of methylation marks as a result of deregulated writer and eraser enzymes has also been described as a hallmark of cancer chemotherapy resistance (Ginno et al., 2020; Romero-Garcia et al., 2020; Feng et al., 2021a; Sun et al., 2021a; Zhao et al., 2021). In this review, we summarise the current insights surrounding the dynamics of m^{5}C and its oxidized derivatives (hm^{5}C, f^{5}C, and ca^{5}C) and their relevance in patho-physiological contexts such as pluripotency, development, differentiation, the DNA damage response, and cancer.

DNA/RNA CYTOSINE METHYLATION

The addition of a methyl group at the carbon-5 position of cytosine in DNA in the CpG dinucleotide context is catalysed by the S-adenosyl-methionine-dependent DNMT methyltransferase family. DNMT1 primarily maintains DNA methylation patterns during replication, while DNMT3A,

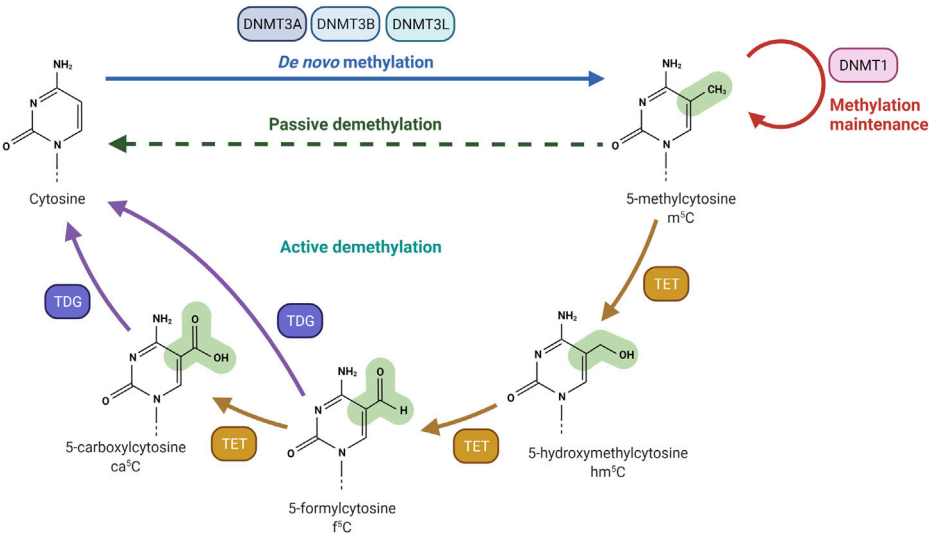

FIGURE 1 | DNA methylation and demethylation machinery. DNA methyltransferase 3A (DNMT3A) and DNA methyltransferase 3B (DNMT3B) are responsible for the creation of DNA methylation patterns. DNA methyltransferase 3L (DNMT3L) interacts and stimulates DNMT3A and DNMT3B methylation activity. DNA methyltransferase 1 (DNMT1) is actively involved in the maintenance of DNA methylation patterns. Passive demethylation occurs in the absence of functional DNMT1, which methylates DNA after each cellular division. Active demethylation is mediated by Ten-eleven Translocation (TET) dioxygenases. TET enzymes oxidize 5-methylcytosine (m^5C) to produce 5-hydroxymethylcytosine (hm^5C), 5-formylcytosine (f^5C) and 5-carboxylcytosine (ca^5C). The glycosylase activity of TDG allows the excision of Tet-produced f^5C or ca^5C nucleobases. Created with BioRender.com.

DNMT3B, and DNMT3L are predominantly involved in the establishment of *de novo* DNA methylation (**Figure 1**) (Lyko, 2018). Proper maintenance of DNA methylation patterns defines the structural and functional identities of cells throughout cell division (Moore et al., 2013). Although DNA methylation patterns are stable, active and passive demethylation modulates a dynamic methylation process (Sadakierska-Chudy et al., 2015). Passive demethylation or replication-dependent dilution occurs after the synthesis of newly replicated DNA strands. Without functional DNA m^5C methylation maintenance of newly synthesized DNA strands, the symmetry of methylation is not re-established and methylation is lost through replication cycles. On the other hand, DNA methylation can be actively reversed by family of α-ketoglutarate-dependent dioxygenases, known as Ten-Eleven Translocation (TET) proteins, which exist as 3 isoforms: TET1, TET2, TET3 (Lan et al., 2020). Hydroxylation of m^5C analogue to 5-Hydroxymethylcytosine (hm^5C), alters the affinity of DNMT1 for the methylated site, and results in loss of the epigenetic mark over several rounds of DNA replication. RNA m^5C modification has been found in mRNAs, rRNAs, tRNAs, and ncRNAs. The RNA specific subset of S-adenosyl-methionine-dependent methyltransferases includes TRDMT1 (tRNA aspartic acid methyltransferase 1) (Li et al., 2021a), also known as DNMT2, and the NSUN1-7 (NOP2/Sun RNA methyltransferases) family, which are responsible for the deposition of m^5C on RNA (Bohnsack et al., 2019; Sun et al., 2019; Liu and Santi, 2000). The m^5C mark is reported to be involved in the regulation of RNA metabolism and is principally associated with structural and functional RNA stability, and its dynamic deposition and removal also permits rapid cellular responses to environmental cues (Gkatza et al., 2019). For example, several lines of evidence identified the m^5C modification as a modulator of the maturation, stability, and translation of mRNA molecules, and is also important for nuclear export (Schumann et al., 2020; Huang et al., 2019). Changes in the m^5C deposition pattern in mRNA is also associated with several hallmarks of cancer including cell survival, proliferation, invasion, and resistance to therapy (Zhang et al., 2020a; Nombela et al., 2021; Zhang et al., 2021; Xue et al., 2020b). Furthermore, the majority of m^5C patterns on RNA are lineage- and tissue-specific (Amort et al., 2017). The presence of the m^5C modification has been described on both the small and large rRNA subunits. M^5C controls ribosome synthesis and processing, and can alter the conformation of the rRNA, effecting translation fidelity (Schosserer et al., 2015; Popis et al., 2016). In tRNA, m^5C is mostly present at the junction of the variable loop and the T-stem spanning positions (47–50) (Van Haute et al., 2019). The presence of m^5C on tRNA has been linked to proper folding of the tRNA molecule into an L-shaped structure. However, m^5C has been also shown to be present at the C38 position in the anticodon loop of tRNA and can modulate the translation fidelity of a specific subset of genes (Huang et al., 2021a). In mRNA, the m^5C modification is enriched in 5′/3′-UTRs, next to Argonaute-binding regions, but is depleted in coding regions (**Figure 2**). m^5C has been also detected in many ncRNAs such as lncRNAs, lincRNAs, pseudogene transcripts, antisense transcripts, and vault RNAs (Amort et al., 2013; Khoddami and Cairns, 2013; Sajini et al., 2019; Sun et al., 2020), and its presence is likely to be linked to processing, stability, and interaction with m^5C reader proteins.

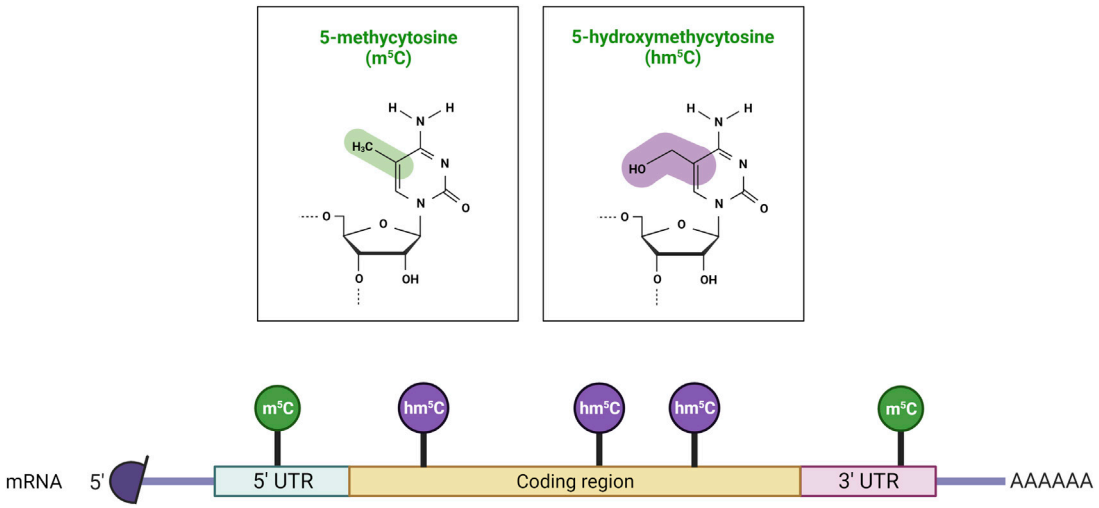

FIGURE 2 | Distribution of m^5C and hm^5C post-transcriptional modifications on mRNA molecules. 5-methylcytosine (m^5C) is predominantly found in 5′ untranslated regions (5′ UTRs), while 5-hydroxymethylcytosine (hm^5C) is present within the coding sequence (CDS). Created with BioRender.com.

5-METHYLCYTOSINE MODIFICATION IN CANCER ONSET

m^5C-driven epigenetic and epitranscriptomic events are entwined in cellular homeostasis. Deregulated m^5C deposition can promote cancer development as a consequence of deregulation of both DNA and RNA molecules (Sun et al., 2021b). The m^5C-mediated upregulation of proto-oncogenes or silencing of tumour suppressor genes, together with an enhanced translation rate and stability of mRNA oncogenes, are common molecular events in many types of cancers including leukaemia, breast, bladder, gastric, ovarian, colorectal, and lung (Atala, 2020; Zhang et al., 2020a; Sun et al., 2020; Hu et al., 2021a; Awah et al., 2021; Huang et al., 2021b; Li et al., 2021b). Furthermore, alterations in DNMTs expression levels or activity have been linked to both hypermethylation of tumour-suppressor genes and hypomethylation of proto-oncogene genes (Nishiyama and Nakanishi, 2021). In addition, overexpression of the RNA m^5C writer NSUN2 (Okamoto et al., 2012; Yi et al., 2017; Cheng et al., 2018; Chellamuthu and Gray, 2020; Xiang et al., 2020; Hu et al., 2021a; Su et al., 2021; Wang et al., 2022) and RNA m^5C reader YBX1, has been associated with mRNA hypermethylation and oncogene activation (Chen et al., 2019). Furthermore, the NSUN2-YBX1-oncogene axis is a commonly deregulated pathway during the cancer onset and progression (Wang et al., 2022).

5-METHYLCYTOSINE MODIFICATION IN DNA DAMAGE RESPONSE

Since several RNA species (DNA damage response RNAs, damage-induced lncRNAs, *de novo* transcripts and DNA:RNA hybrids) have been described to be essential during DNA damage repair (Ketley and Gullerova, 2020), a pioneering study has investigated the possibility of RNA post-transcriptional modification relevance in DNA double strand break (DSB) repair. DNMT2/TRDMT1 protein was shown to be recruited to damage sites, where it is able to catalyse the deposition of m^5C residues onto DNA:RNA hybrids (Chen et al., 2020a). m^5C-modified DNA:RNA hybrids promoted the recruitment of specific readers including Rad52, which drives the later stages of DNA repair. In addition, loss of DNMT2 proved to be detrimental during the DNA damage response, suggesting the importance of m^5C presence during HR-mediated DNA damage repair (Zhu et al., 2021).

TEN-ELEVEN TRANSLOCATION-DRIVEN ITERATIVE 5-METHYLCYTOSINE OXIDATION (5-HYDROXYMETHYLCYTOSINE > 5-FORMYLCYTOSINE > 5-CARBOXYLCYTOSINE) IN DNA AND RNA

The presence of m^5C in the genome is dynamically controlled by the antagonising action of specific writers and erasers. DNMT family members are mainly responsible for the deposition of m^5C at both the DNA and RNA level, while Ten-eleven translocation (TET) proteins are considered m^5C erasers. Active demethylation of m^5C is achieved by TET-mediated sequential oxidation with the production of hm^5C (5-Hydroxymethylcytosine), f^5C (5-formylcytosine), ca^5C (5-carboxylcytosine) analogues (**Figure 1**) (Ito et al., 2011). Then, the N-glycosidic bond of f^5C and ca^5C is processed by the Thymine DNA Glycosylase (TDG) to form abasic sites,

followed by Base Excision Repair (BER) to restore the unmodified cytosine (He et al., 2011; Bordin et al., 2021). Alternative pathways include; 1) direct deformylation (f^{5}C) and decarboxylation (ca^{5}C) mediated by DNMT3A/B (Feng et al., 2021b), 2) AID/APOBEC-dependent deamination and production of 5hmU followed by TDG cleavage (Cervantes-Gracia et al., 2021), and 3) passive DNA replication-dependent loss (Vincenzetti et al., 2019). However, TET enzymes appeared to be involved in the oxidation of both DNA and RNA m^{5}C (Wu and Zhang, 2011; Fu et al., 2014; He et al., 2021a). *In vitro* studies confirmed that double stranded DNA is the preferred TET substrate, followed by DNA:RNA hybrids, single stranded DNA and single stranded RNA. Double strand RNA molecules were shown not to be TET substrates, likely due to TET discrimination against the RNA A-form conformation (DeNizio et al., 2019).

5-HYDROXYMETHYLCYTOSINE

The hm^{5}C mark has been annotated at promoters, enhancers, and in gene bodies (Cui et al., 2020). While hm^{5}C is mainly associated with active gene transcription and an open chromatin structure, its role depends on the genomic context (active vs. poised genes) (Choi et al., 2014). Furthermore, hm^{5}C modification has a locus and tissue specific signature and serves as a feature of cellular state and identity. Genome-wide mapping at a single-nucleotide resolution level using modification specific antibodies, has estimated that around the 5% of cytosine residues in mammalian genome are modified as m^{5}C and less than the 1% are hm^{5}C (He et al., 2021b). The presence hm^{5}C has been described not only at promoters, enhancers and gene body regions, but also on several RNA molecules (Delatte et al., 2016). m^{5}C and hm^{5}C modifications have been identified as stable epigenetic marks, and different chromatin-binding proteins have been shown to specifically bind to either m^{5}C or hm^{5}C, suggesting these modifications have distinct functions in epigenetic regulation. Recent studies have identified several proteins which preferentially "read" m^{5}C or its oxidized forms. For example, the Methyl-CpG binding domain (MBD) protein family plays a pivotal role in determining the transcriptional state of the epigenome and shows a strong preference for hm^{5}C over the m^{5}C modification (Buchmuller et al., 2020). MBD proteins mainly belong to chromatin-bound repressor complexes, which coordinate crosstalk between m^{5}C methylation, histone modifications, and chromatin organization. TET-mediated hm^{5}C biogenesis blocks the reader function of the MBDs and alleviates their transcriptional repression, producing a new platform for hm^{5}C specific readers and transcriptional activation.

5-FORMYLCYTOSINE AND 5-CARBOXYLCYTOSINE

Despite the fact that f^{5}C and ca^{5}C oxidized forms of m^{5}C are considered short-lived intermediates in the demethylation process, and their steady-state levels are many orders of magnitude lower than hm^{5}C, emerging evidence indicates that f^{5}C and ca^{5}C might have independent epigenetic signalling roles in recruiting modification-specific "reader" proteins (Song and He, 2013). f^{5}C and ca^{5}C can be recognized by transcriptional regulators, DNA repair factors, and chromatin regulators, predominantly stimulating gene activation. Beside these regulatory roles, some studies have proposed the TET-mediated oxidative products f^{5}C and ca^{5}C are mutagenic bases that can threaten the genomic integrity if not properly eliminated. Moreover, deregulation of TET-TDG-BER pathway and inefficient f^{5}C and ca^{5}C clearance has been linked to DNA damage and the production of DSBs (Weber et al., 2016).

5-HYDROXYMETHYLCYTOSINE MODIFICATION IN PLURIPOTENCY, DEVELOPMENT, AND DIFFERENTIATION

Transcriptome flexibility is required for embryonic stem cell (ESC) differentiation (Dawlaty et al., 2014; Lan et al., 2020). Cell fate commitment requires efficient and timely control of the expression of pluripotency-associated factors. hm^{5}C can promote a rapid response during differentiation processes (Cimmino et al., 2011; Kuehner et al., 2021). The RNA hm^{5}C modification has been described as a guardian of the transcriptional landscape, able to regulate the balance between pluripotency and lineage-priming mRNAs and ensures ESC differentiation in a timely and orderly manner (Wu et al., 2018; Yang et al., 2020). TET-dependent hydroxymethylation of mRNA molecules can regulate RNA half-life and splicing. In mouse embryonic stem cells (mESCs) hm^{5}C has been linked to the downregulation of certain mRNAs linked to pluripotency allowing ESC-to-EB (embryo body) differentiation (Lan et al., 2020). During somatic reprogramming to pluripotency, hm^{5}C deposition drives site-specific demethylation of reprogramming enhancers and promoters resulting in the formation of iPSC (induced pluripotent stem cells) (Caldwell et al., 2021). TET triple knockout cells failed to undergo iPSC reprogramming, highlighting the importance of m^{5}C hydroxylation during state transitions. Furthermore, a report on somatic reprogramming determined the contribution of hm^{5}C deposition decoupled from the production of the oxidative derivatives f^{5}C and ca^{5}C using a hm^{5}C-stalled TET enzyme. Interestingly, hm^{5}C deposition alone is not sufficient for iPSC reprogramming, and f^{5}C, ca^{5}C and the TDG protein appeared to be crucial in this process, suggesting a different role of hm^{5}C and the f^{5}C and ca^{5}C epigenetic signatures (Caldwell et al., 2021).

5-HYDROXYMETHYLCYTOSINE MODIFICATION IN CANCER ONSET

hm^{5}C has been proposed to act as a novel diagnostic and prognostic marker in several human malignancies (Scourzic et al., 2015; An et al., 2017). Loss or inactivation of TET

enzymes and deregulation of m^5C demethylation are emerging as crucial determinants for cancer phenotypes. Modulation of the expression and activity of TET proteins can occur as a result of different mechanisms, such as m^5C-mediated silencing of the TET loci or changes in the intracellular concentration of TET co-factors (i.e., α-Ketoglutarate, oxygen, iron, vitamin C) (Scourzic et al., 2015; Yue and Rao, 2020; Matuleviciute et al., 2021). Several studies have outlined a negative correlation between the loss of TET activity with increased levels of m^5C and decreased levels of hm^5C, and poor prognosis in cancers including lung, cervical, breast, glioblastoma, and hematopoietic (López-Moyado et al., 2019; Gao et al., 2021; Xu et al., 2021; Lopez-Bertoni et al., 2022; Xu et al., 2022). The deregulation of m^5C and hm^5C affects cell transcriptional programs and leads to cancer stem-like phenotypes. Moreover, aberrant deposition of hm^5C has been also proposed as a contributing factor to chemotherapy resistance (Kharat and Sharan, 2020).

5-HYDROXYMETHYLCYTOSINE MODIFICATION IN THE DNA DAMAGE RESPONSE

Of note, TET-mediated hm^5C accumulation has also been described at DNA damage, suggesting a crucial role for hm^5C in promoting DNA damage repair (Kafer et al., 2016). Possible mechanisms of action could be linked to the ability of hm^5C to promote or maintain an open chromatin configuration. hm^5C modification is able to control DNA accessibility through a DNA-end breathing motion that can decrease nucleosome affinities, facilitate RNA polymerase II elongation, and lower the thermodynamic stability of the DNA duplex (Mendonca et al., 2014; Li et al., 2022). Local accessibility of hm^5C chromatin driven by hm^5C, could serve as a platform for the recruitment of late-acting DNA damage repair factors. The hm^5C modification has also been found to promote the formation of DNA:RNA hybrids (R-loops) *in vitro* and *in vivo* (Sabino et al., 2022; Shukla et al., 2022; Yang et al., 2022). Given that DNA:RNA hybrids are well-established triggers of DNA damage, hm^5C modification has been proposed as novel player in genome instability. Several studies have documented a direct association between TET deficiency and increased level of DNA double strand breaks caused by the accumulation of R-loop structures. Ineffective m^5C demethylation can further impair DNA damage repair through the retention of m^5C readers on the R-loops and delayed R-loop resolution. Even if hm^5C deposition in DNA damage responses is not fully understood, a direct correlation between the level of hm^5C and fork stability has recently been described (Kharat et al., 2020). Upon DNA damage, ATM and ATR kinases can phosphorylate TET proteins and stimulate hm^5C deposition close to the replication fork. The excessive presence of hm^5C at replication forks triggers BER-mediated repair of hm^5C, leading to the production of abasic sites which could sources of genome instability.

BEYOND 5-METHYLCYTOSINE: THE ROLE OF N6-METHYLADENOSINE MODIFICATION

Dynamic and reversible chemical control of DNA and RNA also encompasses other methylated nucleobases. So far, several enzymatically methylated residues including N6-methyladenosine (m^6A) (Zhu et al., 2020), N1-methyladenosine (m^1A) (Xiong et al., 2018), N7-methylguanosine (m^7G) (Enroth et al., 2019) and N4-methylcytosine (m^4C) (Chen et al., 2020b) have been described in all major RNA types, while their presence in DNA is mainly restricted to m^6A (Xu and Bochtler, 2020). For example, the existence of m^6A in DNA has been suggested to be dependent on RNA catabolism rather than a specific m^6A writer, arguing against the proposed role of m^6A as a heritable epigenetic mark (Musheev et al., 2020). This evidence is also corroborated by the homogeneous distribution of m^6A throughout the genome, implying an incorporation of m^6A form the RNA nucleoside pool rather than from the direct action of a DNA methyltransferase. Although some reports have proposed a role for m^6A in the epigenetic control of the heterochromatin formation, m^6A-mediated regulation of chromatin dynamics appears to depend more on m^6A methylated RNAs such as chromosome-associated regulatory RNAs (carRNAs) (Zhang et al., 2020b; Liu et al., 2020; Selmi and Lanzuolo, 2022) rather than a direct effect of m^6A deposition in the genome. Nevertheless, m^6A is a well-established RNA epitranscriptomic mark and its dynamical and reversible write and erase processes are mainly regulated by some members of the methyltransferase-like gene family (METTL3, METTL13 and METTL14) and FTO and ALKBH5 enzymes, respectively (Meyer and Jaffrey, 2014). The m^6A writing process predominantly occurs in the nucleus, while reading and erasing events are reported in both the nucleus and cytoplasm. Distinctive subcellular localization of m^6A erasers and readers confers the specific roles of the m^6A residue, and thereby influences different pathways. m^6A functions have been linked to several biological processes such as modulation of splicing by altering the structure of pre-mRNAs (Zhou et al., 2019), increased miRNA biogenesis by enhancing the recognition and processing of the microRNA microprocessor complex protein DGCR8 (Han et al., 2021), transcription termination by facilitating the co-transcriptional R-loops formation (Yang et al., 2019), regulation of DNA damage repair by the accumulation of DNA:RNA hybrids at DSB sites (Zhang et al., 2020c; Qu et al., 2021) development and pluripotency (Zhang et al., 2020b; Jin et al., 2021). Furthermore, it has been reported that m^6A modification can influence mRNA export from the nucleus, determine transcripts turnover and stimulate translation initiation (Jiang et al., 2021). Perturbation of m^6A deposition and elimination dynamics, due to upregulated or mutated enzymes, has been associated with tumor initiation and progression, metastasis, cell proliferation and self-renewal (He et al., 2019; Zhou et al., 2020). Moreover, opposite effects of m^6A modification levels reported in some cancer settings (e.g., ovarian cancers), can be explained by the involvement of specific m^6A readers in the stability of either oncogene or tumor-suppressor m^6A-modified mRNAs (Chen et al., 2021b; Huang et al., 2022).

CONCLUDING REMARKS

A growing body of evidence suggest that epigenetic and epitranscriptomic dynamics are profoundly interconnected. Methyltransferases ("writers") and demethylases ("erasers") functionally cooperate and compete to maintain the appropriate amount of m^{5}C and its oxidized derivatives across both the genome and the transcriptome. Maintenance of effective, time regulated, and lineage-specific methylation-demethylation dynamics is needed for cellular homeostasis and responses to diverse stimuli. Mutations in epigenetic and epitranscriptomic modifiers can deregulate normal cellular differentiation and programmed growth control. As a consequence of altered patterns of hm^{5}C and m^{5}C, mainly characterised by global hypomethylation and focal hypermethylation, multiple stages of tumorigenesis including initiation, progression and metastasis, are promoted. Moreover, numerous reports correlate the loss of hm^{5}C with poor prognosis. Restoration of proper methylation-demethylation dynamics in cells could be achieve with several approaches. Identification of druggable targets of both main players (DNMTs, TETs) and pivotal intermediates (miRNAs or lncRNAs) is crucial for the development of classical inhibitors or RNA-based drugs. A combination of epigenetic therapy and classical chemotherapy is a promising approach aiming at reducing tumour growth and self-renewal characteristics, while reducing chemoresistance (Hu et al., 2021b).

In addition, the intriguing possibility of precise and synchronized crosstalks between diverse epigenetic and epitranscriptomic marks (e.g., m^{5}C and m^{6}A) (Rengaraj et al., 2021) as versatile checkpoints in the maintenance of cellular homeostasis, may underpin a complex and comprehensive landscape of the cellular methylation game.

AUTHOR CONTRIBUTIONS

AA and MG discussed the structure and content of the review. AA wrote the draft and MG edited the draft.

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SUPPLEMENTARY MATERIAL

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