Chapter

DNA Hydroxymethylation in the Regulation of Gene Expression in Human Solid Cancer

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Abstract

Chromatin in cancer undergoes chemical and structural changes that alter gene expression patterns. One of the chemical modifications that impacts gene regulation is 5-hydroxymethylcytosine (5hmC), also called DNA hydroxymethylation. 5hmC is a stable mark that is commonly associated with transcriptional activation. In cancer, the global loss of 5hmC is a hallmark. In addition, the deregulation of 5hmC in specific regions of the genome, such as enhancers, promoters, and body of the gene, alters the expression of genes in cancer. These alterations have been detected by the improvement in the mapping of 5hmC at genomic scale, which has allowed us to evaluate the sites where 5hmC alterations occur and the genes that are affected. In this chapter, the recent knowledge about the status of 5hmC in genome specific sites of human solid cancers, the relationship with enzymes ten-eleven translocation (TET) and **isocitrate dehydrogenase** (IDH) involved in the dynamic regulation of 5hmC levels, and the impact of the 5hmC aberrant changes on the genic expression in these malignances is reviewed.

Keywords: DNA hydroxymethylation, 5hmC, cytosine modifications, DNA demethylation, epigenetics, chromatin, gene expression, cancer

1. Introduction

In carcinogenesis, genetic alterations are necessary along with the deregulation of the epigenetic phenomena. Epigenetics could be defined as the study of the mechanisms that control gene expression without modifying the DNA sequence [1]. In cancer, epigenetic changes can be used to identify the site of origin of the tumor, detect malignant tumors in the earliest stages, and also allow the identification of more aggressive tumors and predict the response to drug therapy [2, 3]. On the other hand, they can be used as therapeutic targets in epigenetic therapy [4].

DNA methylation is a widely studied epigenetic phenomenon, and it occurs predominantly (80%) in a CpG context, where cytosine (C) is methylated in carbon 5, generating 5-methylcytosine (5mC). 5mC constitutes approximately 1% of all

DNA bases [5] and is associated with the regulation of gene expression, recruitment of transcription factors, nucleosome positioning, splicing, and imprinting. Specifically, in cancer, the overall decrease of 5mC is a general brand and is enriched in specific areas such as the promoter and the first exon, which generates gene silencing and is associated with the development of cancer [6]. Although 5mC is a stable mark, 5mC was shown to oxidize to 5-hydroxymethylcytosine (5hmC). In mammals, the conversion of 5mC to 5hmC is catalyzed by members of the ten-eleven translocation family (TET1, TET2, and TET3), α-ketoglutarate (αKG), and Fe^{2+}-dependent dioxygenases. TET enzymes also oxidize 5hmC to 5-formylcytosine (5fC) and 5-carboxycytosine (5cC) [7].

Currently, it is reported that DNA hydroxymethylation is a stable epigenetic mark that the cells can inherit to its daughter's cells rather than just a transition state [8]. This mark represents the second most abundant C variant in the mammalian genome, always at levels below 5mC, but on the other hand, the 5hmC is 10–100 times higher than the 5fC and 5cC [9, 10].

In mammals, 5hmC occurs almost always (99.89%) in a CpG context [11] and, interestingly, is more enriched in distal regulatory elements of the promoter (46.4%), the body of the gene, and near the cis elements of transcription factors and is less abundant in the promoter region [9–11], suggesting that the hydroxymethylation of DNA has an extensive function in gene regulation. In addition, the enrichment of 5hmC in the different functional elements has been associated with gene transcriptional activation [9].

In humans, the presence of 5hmC would vary significantly between tissues: in the brain (0.67%), rectum (0.57%), liver (0.46%), colon (0.45%), and kidney (0.38%), the 5hmC levels are high, while in the lung (0.14%), they are relatively low and very low in the heart (0.05%), breast (0.05%), and placenta (0.06%) [12]. Compared to normal tissue, many solid neoplasms (e.g., breast, colon, prostate, and melanoma) are characterized by the overall loss of 5hmC. In some isolated cases of cancer, it was reported that 5hmC usually increases; however, the general trend is a global decrease of 5hmC in carcinogenesis [13]. Importantly, hypo-5hmC in cancer occurs in the body of the gene, enhancers, and near cis elements of transcription factors, altering gene expression [14, 15]. However, so far, the role of hydroxymethylation of DNA in cancer biology is not completely clear, and more studies are needed that provide deeper information on functions or potential applications as biomarkers. The purpose of this chapter is to provide current knowledge of the deregulation of 5hmC in genome specific sites, the relationship with enzymes ten-eleven translocation (TET) and **isocitrate dehydrogenase** (IDH) involved in the dynamic regulation of 5hmC levels, and its impact on gene expression in different human cancers.

2. 5hmC status in solid cancer

2.1 5hmC status in melanoma

Melanoma is a type of melanocyte neoplasm that is considered highly aggressive [16]. As one of the most aggressive human tumors, it can perform distal and lethal metastases despite the volume of the tumor being 1 mm^3 [17].

Melanoma is a complex disease influenced by genetic and epigenetic alterations. Importantly, epigenetic phenomena in this tumor include hypermethylation of the phosphatase and tensin homolog (PTEN) promoter and $p16^{ink4}$, which is associated with the silencing of tumor suppressor genes [18, 19]. In addition, repressive chromatin marks that silence the TGF-pathway have been reported [20]. On the other

hand, the increase in chromatin-modifying enzymes, such as Ezh2 methyltransferase, has been published [21]. A significant decrease in members of the TET enzyme family has been reported, which correlates with low global levels of 5hmC [17–22]. Thus, altered patterns of 5hmC in melanomas have been observed, and in this sense, the analysis of 5hmC in melanomas showed a hypo-hydroxymethylation in the body of the Ras-related C3 botulinum toxin substrate 3 (RAC3) gene, the type 1 insulin-like growth factor receptor (IGF1R), and tissue inhibitor of metalloproteinases 2 (TIMP2) (**Table 1**). However, the effect at the expression level was not determined. In two studies published independently, they reported that in melanoma the expression levels of IGF1R and TIMP2 are high [23, 24], suggesting that hypo-5hmC in the body of the gene probably contributes to the high expression of the IGF1R and TIMP2 gene. On the other hand, it has been observed that the overexpression of TIMP2 in B16F10 melanoma cells reduces invasion and angiogenesis and inhibits apoptosis [24].

2.2 5hmC status in glioma cancer

Gliomas are the most common brain tumors, being classified by grades (I–IV), based on differentiation status, malignant potential, response to treatment, and patient survival rate. Grades III and IV are referred as high-grade glioma and have the worst prognosis with a median survival for grade III of 2–3 years, while grade IV, named glioblastoma (GBM), has approximately 15 months [45]. In 2016, the

Name of gene	5hmC status	5hmC variable position	Effect	Target cancer	Refs.
RAC3, IGF1R, TIMP2	Hypo-5hmC	Gene body	ND	Melanoma	[17]
SOX2-OT, CHD2	Hyper-5hmC	TSS1500	Activation	Glioma	[25]
LSMEM1	Hyper-5hmC	5′UTR	Activation	Glioma	[25]
v-myc, FAM49A, DDX1, IL-2, IL-15, PRC2	Hyper-5hmC	ND	Activation	Neuroblastoma	[26, 27]
PTEN, hMLH1, IRX1	Hypo-5hmC	Promoter	Repression	Gastric	[28]
GATA6, MMP11, VAV2, LATS2	Hyper-5hmC	Promoter and gene body	Activation	Pancreas	[29]
TBX15	Hyper-5hmC	Promoter	Activation	HCC	[30]
COMT, FMO3, LCAT	Hypo-5hmC	Promoter	Repression	HCC	[30]
CCNY, CDK16	Hyper-5hmC	Loci and promoter	Activation	HuRCSC	[31]
VHL, SETD2	Hypo-5hmC	Gene body	Repression	ccRCC	[14]
CA2, FMN2, PDCD4, PKIB, SLC26A2, ALOX15*, GHRHR*, TFP12*, TKTL1*	Hypo-5hmC	Loci, promoter	Repression	Colon	[32–42]
TESC, TGFBI, BMP7, NKD2	Hyper-5hmC	Loci	Activation	Colon	[32]
GLO1	Hyper-5hmC	Promoter	Activation	Endometrial	[43]
LZTS1	Hypo-5hmC	Loci	Repression	Breast	[44]

**The effect of 5hmC was not determined in these genes. 5hmC, 5-hydroxymethylcytosine; TSS1500, 1500 bases upstream transcription start site; 50 UTR, 50 untranslated region; HCC, Hepatocellular carcinoma; HuRCSS, Kidney renal stem cells; ccRCC, Clear cell renal cell carcinoma; ND, not determined.*

Table 1.
Genes with aberrant 5hmC in different solid human neoplasms.

World Health Organization (WHO) introduced a new classification where molecular markers were taken into account. In this classification glioma are divided into subtypes based on the isocitrate dehydrogenase 1 (IDH1) gene mutation status [46]. IDH can affect DNA hypermethylation at certain promoter regions, resulting in a glioma CpG island methylator phenotype [47]. In glioma, it has been described as an aberrant 5mC status in CpG island shores and is 5hmC-dependent, and it correlates with disease progression [15]. Moreover, in a previous report, the 5hmC patterns were analyzed in GBM samples. They observed a 3.5 reduction in the total 5hmC content and of what was present, localized primarily in super-enhancers and cis elements of transcription factors associated with proliferation. Also, they observed a significant enrichment of 5hmC sites in active transcribed genes in GBM. They reported a total of 2121 active transcribed genes of which 146 have the highest proportion of 5hmC.

As an example, genes with hyper-5hmC in 1500 bases upstream of the transcription star site (TSS1500) that are transcriptionally active are SOX2 overlapping transcript (SOX-OT) and chromodomain-helicase-DNA-binding protein 2 (CHD2) (**Table 1**). Additionally, the gene that encodes the leucine-rich single-pass membrane protein 1 (LSMEM1, also called C7orf53) is transcriptionally active and also presented elevated levels of 5hmC in the 5′ untranslated region (5′UTR) (**Table 1**) [25].

2.3 5hmC status in pediatric embryonal tumors

The pediatric embryonal tumors are a rare type of childhood cancers that derive from neuroectodermal tissue and share related histopathological features despite distinct anatomical locations and diverse clinical outcomes [48]. These tumors can originate in many parts of the body, the ones that are derived from the sympathetic nervous system are called neuroblastoma, and the ones that are derived in the brain are called medulloblastoma [49].

2.3.1 Medulloblastoma

Medulloblastoma (MB) is the most common malignant brain tumor of childhood, the overall 5-year disease-free survival remains low (36%) for patients with dissemination, and prognosis remains poor for patients with recurrent MB [50]. In addition, majority of survivors exhibit long-term neurocognitive and neuroendocrine complication as a result of therapy [51, 52].

The analysis of the 5hmC levels in MB showed a reduction, in comparison to non-neoplastic cerebellum [53]. This finding agrees with other reports where the loss of 5hmC is a common event in other brain tumors as well as tumors of different origins [54]. With this discovery, the expression of TET1, TET2, TET3, IDH1, and IDH2 was analyzed, but the profile could not explain the reduction of 5hmC [53], although the difference in expression of this genes did correlate with the different MB molecular subgroups, suggesting a possible role for TET and IDH genes in the control of specifically developmental pathways activated in MB subgroups. This inability to associate the overall reduction of 5hmC levels and the expression of TET and IDH genes could be due to the method of selection of the 5hmC screening and the lack of analysis of the genomic distribution of 5hmC.

2.3.2 Neuroblastoma

Neuroblastoma is accountable for more than 7% of malignancies in patients younger than 15 years and is responsible for 15% of all pediatric oncology deaths.

Risk assessment based on several clinical and biological features, including age, stage, avian myelocytomastosis viral oncogene (v-myc) status, ploidy, and histology, classified the patients into three groups, low-risk (LR), intermediate-risk (IR), and high-risk (HR) disease [26]. Comparison of the 5hmC profile in LR vs. HR permitted the identification of 3320 genes with differential 5hmC levels between the groups. In the LR group, genes with 5hmC enrichment and increased expression were v-myc, family with sequence similarity 49 member A (FAM49A), and DEAD-box helicase 1 (DDX1) (**Table 1**). Regarding the HR group, the genes with hyper-5hmC and high expression include genes involved in inflammation (IL-2 and IL-15) and in the polycomb repressive complex 2 (PRC2) [27].

2.4 5hmC status in parathyroid cancer

Parathyroid cancer is a rare, indolent, and slowly progressive tumor, being the rarest cause of primary hyperparathyroidism. Surgery is the option of treatment; thus the early identification in the preoperative period is vital [55]. Clinical characteristics of parathyroid cancer can overlap with benign parathyroid disease [55, 56]. Barazeghi E. et al. showed reduced global levels of 5hmC in samples of parathyroid carcinoma compared with samples of normal tissues as well as benign parathyroid adenomas; thus, it was suggested that 5hmC level could be a marker to differentiate between benign and malign tumors [56]. Analysis of TET protein expression indicated variable expression of TET1 in parathyroid adenomas and carcinomas, and additionally reduced or absent expression of TET2 was observed in parathyroid carcinomas as compared with normal parathyroid tissue in concordance with the reduced levels of 5hmC reported in parathyroid carcinoma [56, 57]. Furthermore, increased levels of methylation in promoter CpG islands from TET2 were reported [57]. Analysis of genes regulated by hydroxymethylation on this cancer has not been reported, yet.

2.5 5hmC status in thyroid cancer

Thyroid cancer is the most common tumor of the endocrine organs, accounting for 90% of endocrine tumors. In general, thyroid cancer is originated of follicular cells being divided in papillary thyroid carcinoma and follicular thyroid carcinoma; a reduced percent of thyroid cancers is generated from parafollicular C cells being classified as medullary thyroid carcinomas [58]. The information about 5hmC status is extremely limited, but it has been observed that levels of 5hmC are reduced in papillary thyroid carcinomas as compared with control tissue [59]. However, information about expression of TET proteins and regulation by hydroxymethylation or target genes in this cancer or its subtypes is absent.

2.6 5hmC status in oral cancer

Cancer of the oral cavity is the most common tumor worldwide, the squamous cell carcinoma being the most common histopathology type [60]. A decreased expression of TET2 and a reduction of 5mhC levels in samples of oral squamous cell carcinomas have been reported compared with healthy oral tissues by immunohistochemistry [61]. Also, 5hmC levels decreased progressively from benign oral mucosal lesions to oral squamous cell carcinoma [62]. Expression analysis of target genes regulated by 5hmC or mechanisms implicated have not been reported yet.

2.7 5hmC status in gastric cancer

Gastric cancer is the third cause of cancer death. Environment factors, infections, and genetic and epigenetic alterations are related with development of this cancer [63]. Decreased expression of TET1 mRNA and protein has been reported in gastric cancer, while the expression of TET2 and TET3 did not show differences in expression compared with control tissue. In addition, diminished expression of TET1 has been associated with decreased levels of 5hmC in the promoter of PTEN, human mutL homolog 1 (hMLH1), and iroquois homeobox 1 (IRX1), correlating with their reduced expression levels (**Table 1**) [28]. Thus, reduced expression of TET1 and decreased levels of 5hmC in gastric cancer could be related with the decreased expression of suppressor tumors genes. An in-depth analysis of genes regulated by hydroxymethylation on this cancer could allow new therapeutic strategies.

2.8 5hmC status in pancreatic cancer

Pancreatic cancer is a disease with high mortality rate, being the fourth cause of cancer-related deaths in the United States and most developed countries. Different types of pancreatic cancer can rise; however, the subtype termed pancreatic ductal adenocarcinoma (PDAC) is the most common, which accounts for about 85–90% of cases [64, 65]. The absence of early detection methods, delay in diagnosis, and unsuccessful treatments contribute to the high mortality of this cancer [65].

Low global levels of 5hmC have been reported in pancreatic cancer cell lines and in samples of human tumors compared with healthy pancreatic cells [29]. The reduced levels of 5hmC in samples of human pancreatic tumor tissues correlated with the decreased expression of TET1 [66]. A redistribution of 5hmCs was observed in pancreatic cancer, with enrichment in genomic specific regions as promoters and gene body, particularly of the transcriptional factor GATA6, matrix metallopeptidase 11 (MMP11), vav guanine nucleotide exchange factor 2 (VAV2), and large tumor suppressor kinase 2. The enrichment of 5hmC in this genes is in accordance with the increased expression in human samples of pancreatic cancer [29]. Additionally, it has been suggested that pancreatic cancer patients with high GATA6 survive longer so that GATA6 was proposed as a prognosis marker [67]. On the other hand, high levels of MMP11 were associated with poor prognosis of pancreatic cancer patients [68].

2.9 5hmC status in hepatocellular cancer

Globally, hepatocellular carcinoma (HCC) is the sixth most prevalent cancer and the third leading cause of cancer-related death, estimated to cause the death of 500,000–600,000 people per year [69]. The factors that contribute to mortality in HCC are the 5-year recurrence rates standing at 70% after tumor resection and 15–30% posttransplant [70].

Hepatic B virus (HBV) and hepatitis C (HCV) are the main cause of HCC. For HCC related to HBV, epigenetic alterations play vital roles in hepatocarcinogenesis through direct and indirect mechanisms initiated by HBV [69].

Low global levels of 5hmC have been reported in HCC [69]. A redistribution of 5hmCs was observed in HCC, with an enrichment in specific genomic region as promoters. In this same report, a decrease in the levels of hydroxymethylation was observed in the promoter of different genes, this catechol-O-methyltransferase (COMT), the flavin-containing monooxygenase 3 (FMO3), and lecithin-cholesterol acyltransferase (LCAT) [30] (**Table 1**). The gene T-Box transcription factor 15

(TBX15) with enrichment in the 5hmC promoter region was associated with transcriptional activation, while those that presented a decrease of 5hmC were low [30].

2.10 5hmC status in renal cell carcinoma (RCC)

RCC is the most common parenchymal neoplasm in adults. Among urogenital tumors, it is the second with the highest incidence, which represents 2 to 3% of cancer in humans and 80 to 90% of kidney neoplasms. The most common subtypes of RCC are clear cell carcinoma (ccRCC) and papillary carcinoma [31].

Recently, it was reported that kidney cancer and ccRCC have global decreased levels of 5hmC with respect to normal tissue [14]. Paradoxically in kidney renal stem cells (HuRCSC), hyper-5hmC patterns were detected in specific regions such as the cyclin Y (CCNY) promoter and loci and cyclin-dependent kinase-16 (CDK16) (**Table 1**), which was associated with a transcriptional activation. Likewise, the increased levels of TET1 in HuRCSC were detected, which probably explains the increase of 5hmC in HuRCSC [31].

In another study in which ccRCC was analyzed, it was shown that low levels of 5hmC in the body of the von Hippel–Lindau gene (VHL) and SETD2 methyltransferase are associated with low expression [14] (**Table 1**). In ccRCC loss of SETD2 is associated with genomic instability, aberrant transcriptional program, RNA processing defects, and impacts on cell proliferation, differentiation, and cell death [71]. Additionally, it has been observed that the IDH1 enzyme was significantly downregulated in ccRCC compared to normal kidney cells. Thus, in ccRCC the reduction of IDH1 can be a mechanism for the loss of 5hmC through the downregulation of 2-keto glutarate [14].

2.11 5hmC status in colon cancer

The colon cancer or colorectal cancer is the third most common cancer in the world with more incidence in developed countries [72, 73].

Decreased levels of global hydroxymethylation has been reported in colon cancer [32]. In accordance, decreased expression of TET1 has been reported in samples of human colorectal cancer tissue [33]. Moreover, loss of nuclear expression of TET2 has been observed in colorectal cancer tissue [34]. Thus, aberrant patterns of hydroxymethylation in colorectal cancer has been observed. In this sense, analysis of 5hmC status in colon cancer tissues showed hypo-hydroxymethylation in the promoter region of genes such as:

- Carbonic anhydrase 2 (CA2), which belongs to a group of zinc-binding enzymes, which catalyzes the reversible hydration of CO2 to bicarbonate, that is important for maintenance of pH [32–35]

- Formin 2 (FMN2), involved in cell polarity and cytoskeleton organization and prevents degradation of p21, promoting cell cycle arrest [32–36]

- Programmed cell death 4 (PDCD4), considered a tumor suppressor in colorectal cancer, which can inhibit proliferation and invasion, preventing AP-1 transcription and inhibiting mTOR/Akt [37]

- cAMP-dependent protein kinase inhibitor beta (PKIB), which promotes activation of Akt, contributing to cell growth and proliferation (however, the function of PKIB in colon cancer remains to be determined) [32–39]

- Solute carrier family 26 member 2 (SLC26A2) (low expression of this protein promotes proliferation in vitro observed in colon cancer cell lines) [32–41]
- Arachidonate 15-lipoxygenase (ALOX15)
- Growth hormone-releasing hormone (GHRHR)
- Inhibitor of the tissular factor pathway 2 (TFPI2)
- Transketolase-like protein 1 (TKTL1) (**Table 1**).

Related to 5hmC status and expression, in some cases, the decreased levels of expression of these genes were corroborated. Additionally, hyper-hydroxymethylation and increased expression of different genes have been observed in samples of human primary colon cancer [32–42]. The genes with hyper-5hmC status and high expression include tescalcin (TESC), a calcium-binding protein involved in the promotion of tumorigenesis in colorectal cancer, which activates Akt-dependent NF-κB pathway, promoting proliferation and also contributing to invasion and metastasis in colon cancer [32–42, 74, 75]; transforming growth factor-beta-induced (TGFBI) that promotes cell proliferation, migration, metastasis, and inflammation [32–42, 74–76]; and bone morphogenetic protein 7 (BMP7) that has an augmented expression, but in this case there has been a controversy because in colorectal cancer it has also been reported to have low levels. Additionally, it has been reported that, in colorectal cancer, BMP7 has anti-cancer activity [32–42, 74–78] and naked cuticle homolog 2 (NKD2) [32].

2.12 5hmC status in endometrial cancer

Endometrial cancer is the most common tumor in the genital tract in developed countries [43]. A report showed a decreased level of 5hmC in samples of endometrial cancer by an ELISA-like reaction. In addition, decreased expression levels of TET1 and TET2 mRNA and increased levels of TET3 mRNA were observed [79]. However, in another report, increased levels of TET1 protein and increased levels of 5hmC in endometrial cancer and hyperplasia using immunohistochemistry were indicated [43]. This discrepancy could be associated to the different levels of regulation of TET1 expression. Interestingly, the authors determined that TET1 could promote the accumulation of 5hmC in the promoter of the glyoxalase I (GLO1) gene (**Table 1**), resulting in the increased expression of GLO1 in endometrial cancer [43]. GLO1 promotes proliferation and chemotherapeutic resistance and contributes to progestin resistance used in the treatment of endometrial cancer [43, 79, 80]. Moreover, it was reported that metformin treatment reduced the expression of TET1 and 5hmC levels, promoting the reduction of GLO1 expression and increasing the sensitivity to progestin in a model in vitro [43].

2.13 5hmC status in breast cancer

Breast cancer (BC) is the most frequent neoplasia in women worldwide; data from the WHO suggests that it comprises 16% of the total of cancer cases. In addition, every year 138 million new cases are detected, and approximately 458,000 deaths occur due to the pathology [81].

In breast cancer, low levels of 5hmC in the locus of the gene leucine zipper putative tumor suppressor (LZTS1) (**Table 1**) have been reported. In addition, the level of LZTS1 expression was low in breast cancer samples compared to normal

breast tissue. These results coincide with the low expression of TET1. The results suggest that low levels of 5hmC in the locus of LZTS1 are probably due to the decrease in TET1 [44]. In human cancer, loss of LZTS1 expression has been associated with tumor progression, metastasis, and poor prognosis [82].

3. Conclusion

In the tumors researched in this document, in most of them, an overall global reduction of 5hmC, with accumulation in certain genetic locations or genes, is reported. Some are also related to the overexpression of genes, taking into account that 5hmC is an epigenetic mark of transcription activation. However, the methods used to determine the 5hmC vary between the studies, and not all the methods can be considered reliable to distinguish between 5mC and 5hmC with confidence. Moreover, an area that has not been explored is the effect that 5hmC has on the expression in miRNAs and lncRNA. These observations can be taken to reassess the role of DNA hydroxymethylation status in tumorigenesis.

Expression analyses of TET family members and correlation with 5hmC status have been performed in a wide variety of cancers. However, the biological effect of this aberrant changes in 5hmC levels has not been deeply determined. For example, targeted genes regulated by hydroxymethylation in many cancers have not been established, characterization of the hydroxymethylation patterns in regulator regions of target genes is not determined in some cases, and their expression levels are not corroborated with 5hmC status. Importantly, the effects resulting in the modification or the return to the original state of the hydroxymethylation patterns in cancer are extensively unknown. In addition, since hydroxymethylation can be regulated in different physiological process in health and disease, modifications in 5hmC status could generate undesirable side effects. Thus, more studies are necessary to have a comprehensive understanding of the biological effects and dynamic changes of the hydroxymethylation in cancer, which could allow new therapeutic strategies in the future.

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Conflict of interest

The authors declare no conflict of interest.

Appendices and nomenclature

ALOX15	Arachidonate 15-lipoxygenase
BMP7	Bone morphogenetic protein 7
bHlH	Basic helix–loop–helix
C	Cytosine
CA2	Carbonic anhydrase 2
CCNY	Cyclin Y promoter and loci
ccRCC	Clear cell carcinoma cell renal

CHD2	Chromodomain-helicase-DNA-binding protein 2
CDK16	Cyclin-dependent kinase-16
COMT	Catechol O-methyltransferase
CpG	Islands cytokine phophate guanine
DDX1	DEAD-box helicase 1
DNA	Deoxyribonucleic acid
Ezh2	Histone-lysine methyltransferase
FAM49A	Family with sequence similarity 49 member A
Fe2+	Iron 2+
FMN2	Formin 2
FMO3	Flavin-containing monooxygenase 3
GATA6|	GATA binding protein 6
GBM	Glioblastoma
GHRHR	Growth hormone-releasing hormone
GLO1	Glyoxalase I
G9a	Euchromatic histone-lysine N-methyltransferase 2 (EHMT2)
HIF1α	Hypoxia-inducible factor 1 alpha subunit
HIF2α	Hypoxia-inducible factor 2 alpha subunit
hMLH1	Human mutL homolog 1
HR	High-risk
hypo-5hmC	Hypo-5 hydroxymethylcytosine
HuRCSC	Kidney renal stem cell
H3K36me3	Trimethylation of lysine 36 of histone H3
IDH	Isocitrate dehydrogenase
IDH1	Isocitrate dehydrogenase 1
IDH2	Isocitrate dehydrogenase 2
IGF1R	Insulin-like growth factor 1 receptor
lncRNA	Long noncoding RNAs
IR	Intermediate-risk
IRX1	Iroquois homeobox 1
LCAT	Lecithin-cholesterol acyltransferase
LATS2	Suppressor kinase of large tumors 2
LR	Low-risk
LSMEM1	Leucine-rich single-pass membrane protein 1
MB	Medulloblastoma
mm3	Cubic millimeter
mRNA	Messenger RNA
MMP11	Matrix metalloproteinase-1
MRPL50	Mitochondrial ribosomal protein L50
MYCN	Proto-oncogene, bHLH transcription factor
NDRG3	Neuregulin 3
NKD2	Naked cuticle homolog 2
NF-κB	Nuclear factor kappa B
PCTAIRE1	Serine/threonine protein kinase (PCTK1)
PDAC	Pancreatic ductal adenocarcinoma
PDCD4	Programmed cell death 4
PKIB	CAMP-dependent protein kinase inhibitor beta
PPP1R3A	Protein phosphatase 1 regulatory subunit 3A
PRC2	Polycomb repressive complex 2
PTEN	Phosphatase and tensin homolog
p16ink	Protein 16 ink
RAC3	Ras-related C3 botulinum toxin substrate 3
RCC	Renal cell carcinoma

RNA	Ribonucleic acid
SETD2	SET domain containing 2, histone lysine methyltransferase
SLC26A2	The solute carrier family 26 member 2 (diastrophic dysplasia sulfate transporter)
SOX2-OT	SOX2 overlay transcript
TESC	Tescalcin
TET	Ten-eleven translocation
TET1	Ten-eleven translocation 1
TET2	Ten-eleven translocation 2
TET3	Ten-eleven translocation 3
TFPI2	Tissue factor pathway inhibitor 2
TGF	Transforming growth factor
TGFBI	Transforming growth factor-beta-induced
TKTL1	Transketolase-like protein1
TIMP2	Tissue inhibitor of metalloproteinase 2
TRDN	Triadin
v-myc	Avian myelocytomastosis viral oncogene
VAV2	Vav guanine nucleotide exchange factor 2
VHL	Von Hippel–Lindau gene
5fC	5-Formylcytosine
5cC	5-Carboxycytosine
5mC	5-Methylcytosine
5hmC	5-Hydroxymethylcytosine
5′UTR	5′ untranslated region
αKG	α-Ketoglutarate

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