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REVIEW

Research Progress on the Role of Epigenetic Methylation Modification in Hepatocellular Carcinoma

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Abstract: Hepatocellular carcinoma (HCC) stands as the prevailing form of primary liver cancer, characterized by a poor prognosis and high mortality rate. A pivotal factor in HCC tumorigenesis is epigenetics, specifically the regulation of gene expression through methylation. This process relies significantly on the action of proteins that modify methylation, including methyltransferases, their associated binding proteins, and demethylases. These proteins are crucial regulators, orchestrating the methylation process by regulating enzymes and their corresponding binding proteins. This orchestration facilitates the reading, binding, detection, and catalysis of gene methylation sites. Methylation ences the development, prolisignificantly influferation, invasion, and prognosis of HCC. Furthermore, methylation modification and its regulatory mechanisms activate distinct biological characteristics in HCC cancer stem cells, such as inducing cancer-like differentiation of stem cells. They also influence the tumor microenvironment (TME) in HCC, modulate immune responses, affect chemotherapy resistance in HCC patients, and contribute to HCC progression through signaling pathway feedback. Given the essential role of methylation in genetic information, it holds promise as a potential tool for the early detection of HCC and as a target to improve drug resistance and promote apoptosis in HCC cells.

Keywords: methylation modifying proteins, biomarkers, methyltransferases, HCC tumorigenesis

Introduction

Hepatocellular carcinoma (HCC), the predominant form of liver cancer, presents a significant global health challenge and is the fourth leading cause of cancer-related deaths. According to global cancer statistics, mortality from liver cancer accounts for 8.2% of all cancer-related deaths. The five-year survival rate for HCC and intrahepatic ductal carcinoma is 20.8%.[1–3] Recent advancements in HCC management guidelines have significantly improved monitoring for high-risk groups and optimized tumor staging, leading to innovative approaches for early detection and improved prognosis. Despite the absence of uniform standards, personalized multimodal treatment strategies are increasingly recommended.[3–5]

Epigenetics involves heritable gene expression patterns that do not alter the DNA sequence.[6] The dysregulation of the epigenome becomes a key driver in the early development of HCC.[7] Methylation regulation is an essential component of epigenetics, which is related to embryonic formation. In cancer, it encompassing DNA methylation, RNA methylation, histone methylation, and their respective modifiers. Various enzymes and binding proteins are essential for maintaining different methylation states.[8–10] The combined use of databases with bisulfite detection and sequencing (BSP) for methylation studies is commonly used in experimental research, although some studies have also used techniques like MassARRAYEpiTYPER and Methylation-specific PCR (MSP).[11–13] Recent studies have underscored the potential of reversing epigenetic modifications through modifier inhibitors to restore normal cancer gene expression. This strategy offers promising therapeutic avenues to combat HCC.[14] However, research into epigenetic methylation modification and targeted therapeutic effects in HCC remains largely experimental, with no clinical trial applications reported.

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Aberrant methylation levels may occur in HCC in certain genes associated with the cell cycle, cancer progression as well as the immune environment.[15,16] Targeting these genes induces cancer-specific "synthetic lethality". Such aberrant methylation has proven beneficial for early cancer detection, particularly in HCC.[17] Non-invasive liquid biopsies have been employed to detect epigenetic variants carried by circulating tumor DNA, serving as biomarkers for early HCC detection. Another promising method, enzymatic methyl sequencing (EM-seq), has shown potential to identify early HCC lesions.[18] The experimental results have indicated that certain methylated genes may serve as predictive biomarkers for HCC. The types of methylation modifications of these biomarker genes and their roles in HCC are shown in Table 1. In brief, this comprehensive review aims to encapsulate recent advances in our understanding of epigenetic methylation modifications in HCC at DNA, RNA, and protein levels.

The Role of DNA Associated Methylation in HCC

Regarding DNA methylation, contemporary research has primarily focused on CpG-rich promoters.[31] Additional investigations have examined DNA methylation of other genomic regions.[32] Methylation at the fifth position of cytosine (5mC) is connected with hepatitis B virus (HBV) infection and altered expression of DNA methylation related enzymes.[33] Another common methylation modification, DNA N6-adenine methylation (6mA), observed in human cells, has been linked to tumorigenesis when downregulated.[34] The methylation extent appears to correlate with tumor stage and malignancy.[35] Distinct genes exhibit methylation modifications across different HCC etiologies. For instance, hypomethylation of the F-box protein 43 promoter, glycine dehydrogenase (GLDC) promoter, and signal-transducing adaptor protein-1 (STAP1) promoter is linked to HBV-associated HCC (HBV-HCC).[13,36,37] In hepatitis C-associated HCC (HCV-HCC), demethylation of the transmembrane protein 164 (TMEM164) gene is closely connected.[38] In non-alcoholic steatohepatitis -associated (NASH-HCC), DNA hypermethylation of the zinc finger C3H1 domain-containing protein (ZFC3H1) gene's CpG island influences the cancer's multistage development process.[39]

As the earliest epigenetic alteration, DNA methylation aberrations precede changes in gene expression and the occurrence of diseases, making it significant in the diagnosis and screening of early stage of HCC. Genes associated with the role of DNA methylation modification, such as RDH16,[11] DLC1[12] and STAP1,[13] etc, can be used as biomarkers for early detection of HCC. More detailed reflections of early HCC methylation marker genes and their mechanisms are

Table 1 Methylation-Related Biomarkers in HCC

Biomarker Gene	Methylation Type	Impacts and Mechanisms in HCC
RDH16	DNA methyltransferase inhibitor	HCC migration[11]
DLC1	DNA hypomethylation	Associated with satellite lesions and incomplete tumor capsule[12]
BAIAP2L2	DNA methylation	Potential prognosis and therapeutic target[19]
UBE2	DNA methylation	Diagnostic and prognostic[20]
RPS24	DNA methylation	Cell proliferation and immunosuppression[20]
STAP1	DNA methylation	Diagnostic and prognostic value of HBV-related hepatocellular carcinoma[13]
CCDC50	DNA hypomethylation	HCC prognosis[20]
CKS2	DNA abnormal methylation	Positive correlation with immunological markers[15]
FARSB	DNA hypomethylation	Immune infiltration[16]
m-SEPT9	DNA	Molecular targeted agents treatment efficacy[21]
WHSC1	DNA	Prognosis and therapeutic target[22]
TET1	Demethylation	Immune infiltration and carcinogenesis[23]
AC115619	M6A	Regulation of tumour progression and prognosis[24]
KAA1429	M6A	Sorafenib-resistant hepatocellular carcinoma and HCC tumour metastasis[25]
AURKA	lncRNAs M6A	Prognosis and treatment[26]
GBAP1	M6A	Prognosis indicator and therapeutic target[27]
LncRNA FAM111A-DT	M6A	Proliferation of HCC cells[28]
LncRNA LEAWBIH	M6A	Therapeutic target[28]
LncRNA H19	M5C	Diagnosis and treatment[29]
PRMT3	Histone methylation	Drug resistance[30]

shown in Table 1. Several studies have shown that combining Cell-free DNA (cfDNA) methylation-specific high-throughput or MSP testing with serology enhances the accuracy of serological assays of alpha-fetoprotein (AFP) alone during early screening for HCC.[40]

In targeted therapy of HCC, DNA methyltransferases (DNMT) and DNA demethylases (ten-eleven translocation, TET) in methylation modification may play an important role. In cancer stem-like cells (CSCs), coordinated activation of DNMT3a and TET2 triggers and maintains drug resistance, which would be a therapeutic target for refractory HCC.[41]

Promoter Region Methylation

Methylation at these CpG sites can be categorized into high, low, or demethylation states. Hypermethylation of DNA promoter regions can significantly influence the transcriptional activity of oncogenes or tumor suppressor genes (TSGs), thereby playing a crucial role in tumor recurrence, particularly in advanced tumor stages.[42] The promoter hypermethylation of the gene VIPR1 can suppress the proliferation and invasion of HCC cells through interactions with lncRNA-AC079061.1.[43] Additionally, Promoter hypermethylation affects HCC by modulating the PI3K/Akt or the PI3K/AKT/mTOR signaling pathway.[17,44–46] It also downregulates the Wnt pathway as well as its target genes to suppress cell proliferation in HCC.[47] Promoter hypermethylation of the APC, REPK and UTRN2-1 promoters of oncogenes has been observed to elevate their expression, thereby inhibiting tumor growth, angiogenesis, and invasion, leading to extended relapse-free survival in patients.[30,42,48,49] The hypermethylation status impacts the expression of tumor suppressors or cell proliferation-associated proteins, thereby reducing the development of HCC.[50,51]

Low promoter methylation levels enhance gene self expression.[16,52] Hypomethylation of potential proto-oncogene histone deacetylase 11 (HDAC11) promoters may result in the activation and overexpression of this gen, which leads to the the development of cancer.[53] It has been shown that individuals with lower methylation levels in HCC exhibit shorter survival times and increased vascular involvement.[54] Promoter hypomethylation can positively influence stem cells, fostering the transformation of liver cancer stem cells into malignant cells, which is mainly regulated by DNA demethylases.[55] Hypomethylation of promoters facilitates tumor tissue proliferation, invasion, and migration and serves as an independent prognostic indicator for overall survival and disease progression in liver cancer.[30,54,56] The phenomenon of partial promoter hypomethylation plays a pivotal role in HCC by influencing cell cycle dynamics. For instance, Liu et al have shown that hypomethylation of the Cyclin-D1 (CCND1) promoter, specific to the G1/S phase, enhances the development of HBV-HCC. This discovery posits that it could be a potential diagnostic marker for patients with AFP negative HBV-HCC and AFP-positive chronic hepatitis B (CHB).[57] Besides, hypomethylation of the ZCCHC13 promoter in HCC tissues, leading to its overexpression, promotes cell cycle progression from the G1 phase to the S phase in HCC cells. This aberrant activation is linked with the ATK/ERK/c-MYC/CDK pathway.[58] Futhermore, demethylases have been detected to be overexpressed in HCC cells and liver cancer stem cells, which promote liver fibrosis and reduce radiosensitivity of HCC.[17,55] These findings emphasize the catalytic evoke of promoter hypo- or demethylation on the differentiation of hepatocytes from cancer stem cells (CSCs), thus promoting cell cycle growth and shaping the immune microenvironment.

Promoter methylation is mainly known for its role in gene silencing,[59] although specific cases have shown it can increase gene expression.[48] Both gene silencing and increased gene expression contribute to the initiation and progression of HCC. The review also discusses the influence of DNA methylation in HCC on epithelial-mesenchymal transition (EMT), neoangiogenesis, and the tumor microenvironment (TME). DNMT, a key regulator of DNA methylation, influences the proliferation, migration, and invasion of HCC cells via EMT through its promoter methylation status.[60] Alternatively, DNMT may affect HCC progression by altering its transcriptional activity.[46] EMT is a key mechanism contributing to cancer progression, enhancing the metastatic capabilities of tumor cells through various pathways, including organ fibrosis.[61] Furthermore, some studies further affirming the impact of methylation on tumor-associated angiogenesis.[54] DNA methylation also exerts an influence on immune cells in the TME. Studies have revealed that the hypermethylation of the Natural Killer Group 2D (NKG2D) gene promoter in HCC can markedly affect the disease by modulating immune system responses, thereby establishing its potential as a diagnostic marker for HCC.[62] Notable correlations have been observed between lysyl oxidase-like protein 3 (LOXL3) and methyltransferases. Specifically,

LOXL3 expression is positively correlates with the infiltration of diverse immune cells and the expression of immune checkpoint genes in HCC. High LOXL3 expression has been linked to adverse outcomes in HCC patients.[63] Down regulation of triggered by high methylation evoked by DNMT1, is associated with increased microvessel density (MVD) in HCC.[64] Dysregulated DNA methylation, observed in almost all types of cancer, has been associated with the infiltration characteristics of hepatocellular immune cells, the TME, and its potential impact on immunotherapy, as well as the degree of immune system activation and the prognosis of HCC prognosis.[65] Experimental evidences have demonstrated that promoter methylation modifications of target genes at distinct chromosomal sites may exert disparate effects on HCC (Table 2).

Table 2 Effect of DNA Promoter Methylation Modification of Diverse Genes on HCC

Gene Site	Methylated Target Genes	Promoter Methylation Status	Influence on Self-Expression	Mechanisms on HCC
NC_000001. 11	PIGC	Hyper	Inhibit	An oncogene and its hypermethylation inhibits HCC development[66]
	CDH5	Hyper	Inhibit	Potential tumor suppressor genes, hypermethylation improve the occurrence and development of HCC[67]
	RAB42	Hypo	Promote	A potent prognostic biomarker for HCC[68]
	WASF2	Hypo	Promote	Hypomethylation boosts HCC proliferation, and WASF2 inactivation leads to tumor suppression in vitro[52]
	LINE-1	Hypo	Promote	Hypomethylation can inadvertently lead to the activation of various tumor promoting genes, it identified as an independent prognostic factor for overall survival and disease progression[56,69]
NC_000003. 12	VIPR1	Hyper	Inhibit	VIPR1 transcriptional silencing caused by DNA methylation may contribute to the initiation and progression of HCC[70]
	MUC13	Hypo	Promote	Positive effect on the production of CSCs and promotes the carcinogenesis of liver cells[55]
	HDAC11	Hypo	Promote	Improving HCC progression, related to sorafenib chemotherapy resistance[53]
NC_000004. 12	SFRP2	Hyper	Inhibit	Significantly elevated in poorly differentiated HCC and is an independent risk factor for the development of HCC, it is expected to be used as a non-invasive diagnostic biomarker for HBV-HCC[71]
	CDKL2	Hyper	Inhibit	Hypermethylation status has high specificity and sensitivity for distinguishing adjacent paracancerous tissues from HCC tissues[72]
NC_000005. 10	APC	Hyper	Promote	APC inhibits tumor growth and reduces the proportion of microvascular invasion[48]
	VTRNA2-1	Hyper	Inhibit	The hypermethylation status of the promoter is strongly associated with tumor size, pathological vascular invasion, tumor recurrence, and more advanced tumor stage[42]
	CSF1R	Hypo	Independent of the level of expression	Related to tumor stage and degree of malignancy[35]
NC_000007. 14	STEAP4	Hyper	Inhibit	Inhibits the proliferation and metastasis of HCC cells and mediates the biological effects of HCC cells through the inhibition of the PI3K/AKT/mTOR pathway[44]
NC_000008. 11	ADRA1A	Hyper	Inhibit	Low expression promoter was significantly in connection with alcohol intake, AFP and cirrhosis, but not link to TNM stage[73]
NC_000009. 12	RECK	Hyper	Inhibit	Promoter hypermethylation is linked with HCV-HCC, and varying degrees of methylation are engaged with serum RECK levels, lymph node metastasis, and vascular infiltration[49]
	GLDC	Hypo	Promote	Promote the invasion and proliferation of liver cancer[74]

(*Continued*)

Table 2 (Continued).

Gene Site	Methylated Target Genes	Promoter Methylation Status	Influence on Self-Expression	Mechanisms on HCC
NC_000011. 10	ATM	Hyper	Inhibit	Encouraging HCC development, possibly increasing the risk of HCC by regulating ATM expression[73]
	KCNQ1	Hyper	Inhibit	Tumor suppressor factor, and its expression is reduced to inhibit liver cancer metastasis[75]
	CCND1	Hypo	Promote	Prospective diagnostic marker in patients with AFP-negative HBV-HCC and AFP-positive CHB[57]
NC_000012. 12	USP44	Hyper		Unmethylated in normal tissues, hypermethylated only in HCC tissues[76]
	NKG2D	Hyper	Inhibit	Has good predictive value for HCC diagnosis[62]
	PZP	Hyper	Inhibit	PZP significantly inhibits the proliferation, invasion and migration of HCC cells[77]
NC_000013. 11	PCDH17	Hyper	Inhibit	Regulate EMT and cell proliferation related proteins, influences the biological behavior of HCC and inhibits HCC Proliferation and progression[60]
	SPG20	Hyper		Hypermethylation status is significantly possessing intrahepatic metastasis and results in HCC metastasis[72]
NC_000016. 10	JPH3	Hyper		Tumor suppressors, affect the EMT of HCC cells[51]
	CDH13	Hyper	Inhibit	Tumor suppressor gene, methylation in HCC is significantly increased[78]
NC_000017. 11	Sox15	Hyper	Inhibit	Tumor suppressor genes, down regulation of Wnt pathway and its target gene suppression[47]
	TMEM106A	Hyper	Inhibit	Downward regulated predictions have a poor prognosis, and their levels distinguish between tumor and paracancerous tissues[79]
	SEPT9	Hyper	Promote	Predict MVI and tumor proliferation in HCC, and plasma mSEPT9 can be used as an excellent biomarker for HCC diagnosis and early detection in clinical settings[80,81]
	RNF135	Hyper	Inhibit	Independent predictor of poor prognosis in patients with HCC[82]
NC_000020. 11	NAAT	Hyper		Phosphorylation of LKB3 and PTEN was altered to regulate the PI3K-Akt signaling pathway and promote HCC development[17]
	CTCFL	Hypo		Hypomethylation differs significantly from benign lesion samples, shortened survival, and increased vascular involvement[54]
NC_000023. 11	ZCCHC13	Hypo	Promote	Promotes G1-S transition and promotes cell cycle progression, associated with abnormal activation of ATK/ERK/c-MYC/CDK pathway[58]

Methylation of Other Genomic Regions

In other cancer studies, hypermethylation in intron CpG-rich regions have been observed, potentially leading to oncogenic alterations in tumors due to changes in protein abundance.[83,84] Intron demethylation status in tumour suppressor genes can either amplify or repress the gene, while also regulating the gene's transcript. Histone methylation inhibits or initiates transcription when modifying these genes.[85] LncRNAs may act as transcripts in the intronic regions of genes, where high expression can impact HCC prognosis by mediating metabolic processes.[86] Additionally, the retention of introns (RI) is regulated by protein arginine methyltransferases (PRMTs),[87] and RNA methylation at exon-intron boundaries may impair the splicing of pre-mRNAs.[30] MIR elements in genes and enhancers, sensitive to changes in DNA methylation activity, are implicated in hematological cancers.[88] Currently, the DNA methylation of other regions of these genes in HCC remains an area for further study.

Role of RNA Associated Methylation in HCC

RNA methylation (RM), a dynamically reversible epigenetic modification during translation, involves RNA processing, transport, translation, and metabolism.[89,90] RM mainly includes modifications such as N1-methyladenosine (m1A), 5-methylcytosine (m5C), N3-methylcytidine (m3C), N6-methyladenosine (m6A), and 2'-O-methylation (2′-O-M). These methylation modifications occur at specific positions within RNA base.[91] M6A, m5C, and m1A are associated

with specific gene clusters and risk models in liver cancer.[92] The 2'-O- M of ribosomal RNA (rRNA) regulates autophagy and promotes malignant progression of cancer.[93] RNA methylation is mediated by RNA modifying proteins, including writers (methyltransferases) that catalyze methylation formation, readers (RNA binding proteins) that interpret methylation modification information, and erasers that detect RNA methylation modification. Expression levels of these proteins are significantly upregulated in HCC samples.[79,94,95]

Methyltransferases regulate the interplay between the transcriptome and epitranscriptome during RNA methylation. They facilitate transcriptional dormancy through interconnected mechanisms relevant to the dormancy of adult stem cells and cancer.[96] Furthermore, RNAs can affect gene methylation or directly impact HCC by acting on the methylation level of the corresponding gene.[97–100] RNA methyltransferases (METTs) and RNA-binding proteins (RBPs) offer promising therapeutic targets in HCC. In m6A, METTL3 depends on the YTH domain family 2 (YTHDF2) of RBPs to regulate the proliferation, migration, and invasion of HCC cells. RBPs' YTHDF1 binds NOTCH1 mRNA in the m6A-modified NOTCH pathway to drive HCC stemness and resistance.[41,101] The METT-FTSJ3 induced 2′-O-M process inhibits immune escape and is a promising therapeutic approach.[102] All of these provide new avenues for HCC-targeted therapies.

M6A Modification

The most prevalent RNA methylation modification is M6A, predominantly occurring within the stop codon or 3'UTR of mammalian genes.[91] M6A involves the methylation of the sixth nitrogen atom of adenine This modification is notably prevalent in the mammalian transcriptome.[17] The dynamics of m6A methylation are regulated by various enzymes and RBPs in vivo (Figure 1). The molecular regulatory networks M6A-modulated exhibit differentiable phenotypes in cancer patients. M6A-score models constructed via phenotypic clustering divergences revealed their predictive potential for HCC prognosis and immunotherapy responsiveness, potentially bolstering clinical decision-making or delivering superior prognoses.[103]

Methyltransferases such as METTL3, ZC3H13, and RBPs YTHDF1 and YTHDF2 have been identified to be associated with immune checkpoints and are observed to be overexpressed in HCC.[104] While the expression levels of most m6A-related genes in HCC are significantly higher compared to adjacent non-cancerous tissues, exceptions include zinc finger CCCH type 13 (ZC3H13) and METTL14. The interplay of m6A modification with pathways such as P53, Wnt/β-Catenin, and PI3K/AKT/mTOR may collaboratively influence the tumor microenvironment and immune responses across different HCC clusters.[105–107]

By affecting assorted RNA methylation and acting on multiple cytokine axes, m6A and related regulators promote growth, proliferation and invasion in HCC. In coding RNAs, m6A methylation modifications lead to reduced translational capacity, increased instability, and diminished expression of gene responsive proteins.[108] These alterations can affect the progression of HCC via diverse cell signaling pathways.[105] Among ncRNAs, m6A primarily targets lncRNAs, microRNAs (miRNAs), and circular RNAs (circRNAs). M6A related lncRNAs predicted the prognosis of HCC, in which LncRNA FAM111A-DT and LncRNA LEAWBIH could be used as potential therapeutic targets or diagnostic markers.[109] LncRNAs may affect HCC through the Wnt/β-catenin axis.[110,111] CircRNAs primarily affect the growth, proliferation, and invasion of HCC,[112–114] with circMEMO1 and circRERE, etc, acting as a sponge, functioning with m6A modification, and forming an epigenetic model with positive feedback.[115] Analysis and validation of circRNA-miRNA networks regulated by m6A RNA methylation regulators have revealed that the CircMAP2K4/miR-139-5p/YTHDF1 axis is involved in the proliferation of HCC.[114]

M5C Modification

M5C methylation in human RNA occurs through the transfer of methyl groups to cytosine by methyltransferases, using S-adenosylmethionine as a donor. This process is primarily catalyzed by members of the nucleolar protein family (NOL1, NOL2), the SUN family, and DNMT2 and is integral to RNA stability and functionality.[116] In HCC tissues, the prevalence of mRNA m5C is notably higher compared to the overall level in adjacent tissues. M5C is predominantly enriched downstream of the translation initiation site in the mRNA coding sequence (CDS). Additionally, genes in the Ras pathway, such as GRB2, MAPK3, and PIK3R, exhibit increased m5C in HCC tissues.[117] The expression level of m5C-modified lncRNA H19 is significantly higher in HCC tissues than in non-cancerous tissues. It may contribute to tumor development and progression by recruiting oncogenic proteins and is closely linked to HCC malignancy, making it a potential target or biomarker for HCC diagnosis and treatment.[29]

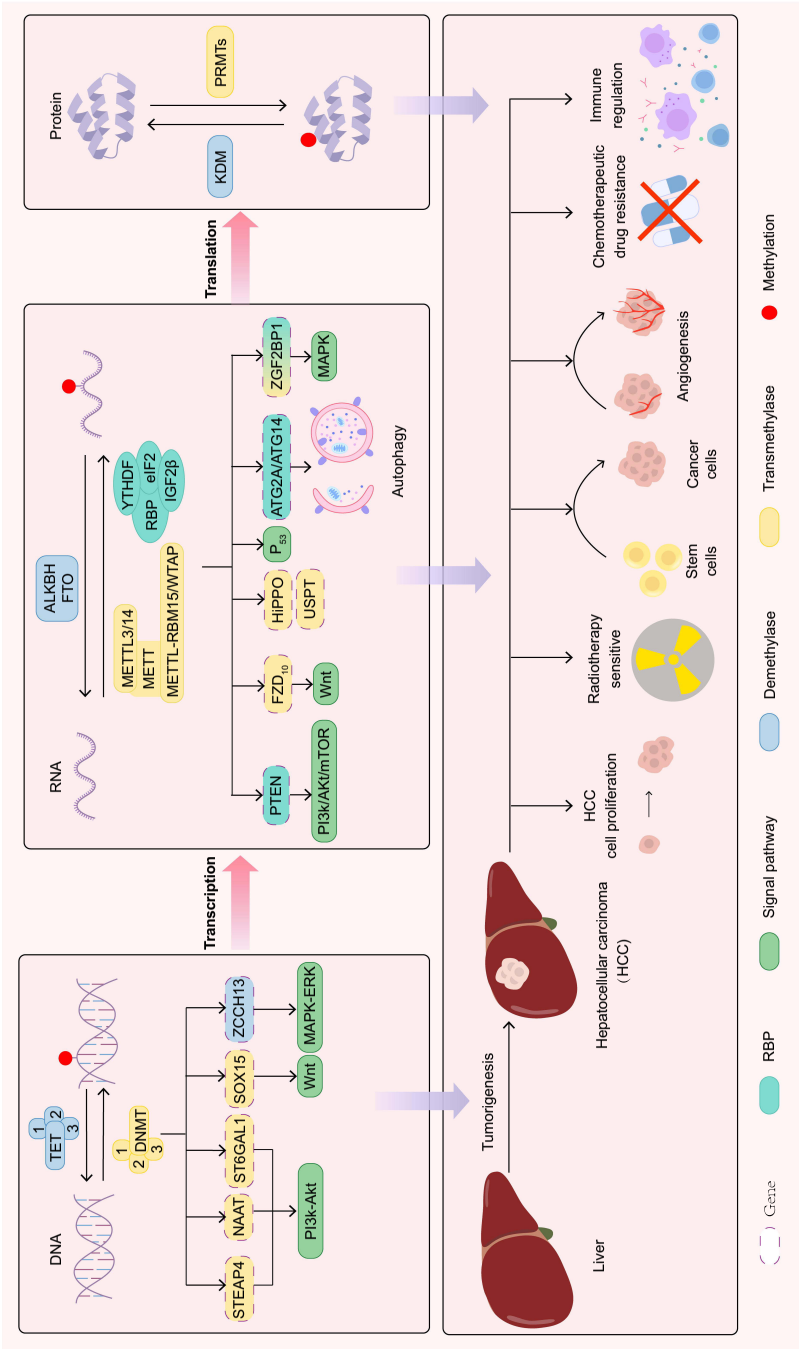

Figure 1 HCC methylation occurs in DNA, RNA, and protein. M6A modifications are regulated by a family of methyltransferases (writer), demethylases (eraser), and specific RNA binding proteins(reader). The methyltransferase family includes METTL family and WTAP. Demethylases include FTO, ALKBH5 meanwhile others, which directly remove m6A modifications from mRNAs. Specific RNA binding proteins consist of YTH domain proteins, the IGF binding protein 2 family, eukaryotic initiation factor 3, and nuclear heterogeneous protein2β1.

M1A Modification

M1A, the methylation of the first nitrogen atom of adenosine in RNA, is a prevalent RNA modification that plays a significant role in tumorigenesis, with tRNA being the most modified class of RNA.[118] The formation of M1A is catalyzed by methyltransferases, with these "writers" specifically recognizing m1A sites as well as inducing downstream effects.[97] Genetic variations in m1A regulators may be linked to mutations in oncogenes, playing a role in the carcinogenesis or metabolic reprogramming of HCC.[90] While M1A regulates the PI3K/AKT/mTOR pathway in gastrointestinal cancers, its specific mechanisms in HCC remain to be fully elucidated.[119] In HCC patient tissues, m1A levels in tRNA are notably increased, and m1A methylation is elevated in CSCs. TRMT6/TRMT61A-mediated m1A methylation is essential for liver tumorigenesis, enhancing m1A methylation in tRNA and activating Hedgehog signaling, thereby driving self-renewal and tumorigenesis in liver CSCs.[120]

Role of Protein Associated Methylation in HCC

Protein methylation comprises two main types: histone methylation and regulator associated methylation, which involve methyltransferases, demethylases, and their corresponding binding proteins. Histone methylation predominantly affects RNA coding and transcription by methylating amino acids at specific sites, thereby influencing the immunophenotype and aggressiveness of HCC. These modification patterns differentially predict TME infiltration, homologous recombination defects (HRD), intratumoral heterogeneity, proliferative activity, mRNA stemness index, and prognosis.[116]

Multimodal Histone Methylation

Histone methylation on chromosome components mainly targets lysine or arginine residues in H3 or H4 histones, with S-adenosylmethionine as the donor molecule for methyltransferase activity.[121] Methylation of specific residues in histones, such as H3K4, H3K27, H3K36, H3K79, H4K20, H3K23, H3K63, and H4K12, plays an important role in RNA regulation.[122] Histone methylation primarily influences RNA coding and transcription through the methylation of amino acids at specific sites, thus regulating the immunophenotype and aggressiveness of HCC.[123]

Histone methylation is diverse and can be classified as monomethylated, bimethylated and trimethylated. Reduced methylation in the H3-lysine monomethylation alteration leads to decreased expression levels of tumour suppressors encoded by the motifs.[26] Dimethylation and trimethylation of H3-lysine related to hypoxia-inducible factor 1α (HIF-1α) activation.[124] Trimethylation at the lysine site is frequently observed with histone H3 lysine 9 trimethylation (H3K9me3) and histone 3 trimethylation at the lysine 27 (H3K27me3) site. Trimethylation reprogramming that has been altered may lead to chromatin compression, gene silence, and transcriptional repression, all of which can encourage EMT and the spread of cancerous cells.[125–127]

Most cancer associated pathway factors with phenotypes are primarily enhanced in the histone H4 hypermethylated group (H4M). H4M modification plays a key role in TME remodeling that is significantly associated with HCC immunophenotyping, while H4M regulators effect HCC epitranscriptome patterns and tumor microenvironment invasion characterization.[123,128] Arginine methylation exhibits the capacity to undergo mono- and bimethylation alterations in response to the of PRMTs.

Role of Histone Methylation Modifiers

Investigations into the effects of histone methylation modulators on HCC have predominantly focused on methyltransferases in conjunction with demethylases. The malignant process of tumourigenesis is frequently accompanied by aberrant modifications of protein arginine methylation, in which PRMTs play a pivotal role. PRMTs comprise a category of methyltransferases that exhibit marked expression alterations in HCC tissues and cell lines, with their isoforms being associated with the sensitivity of specific anticancer drugs.[18] The PARMTs family demonstrates significant correlations with the inflammatory response, glucose metabolism, as well as tumor progression, and is required for the regulation of RNA processing factors, signalling mono- or asymmetric dimethylation.[129,130] PRMTs impose post-transcriptional regulation on retained introns (RIs), whereby their inhibition results in altered splicing rates. By protein arginine methylation, PRMTs regulate the post-transcriptional processing of nuclear retention introns.[87]

Additionally, PRMTs demonstrate a synergistic relationship with the poly ADP-ribose polymerase (PARP DNA repair enzyme), which is responsible for blocking the defective DNA replication induced in response to stress.[131] Type I-PRMTs regulate the intrinsic antiviral immune response by altering RNA splicing. And inhibition of type I-PRMT enhances antitumour immune properties in the refractory setting. Furthermore, arginine methylation further ameliorates oxidative stress by effecting serine levels in HCC patients, thus constituting a potential HCC therapeutic modality.[93]

Histone methylation is also subject to remodeling by Lysine-specific demethylase (KDM, also named LSD). KDM, a protein capable of suppressing the expression of specific genes, regulates cancer progression and is used in anti-cancer therapy for various cancers, including HCC.[132] KDM can repress hepatic CSCs through demethylation in the promoter regions of stemness-associated transcription factors, further affecting stem cell differentiation direction stemness.[133]

In summary, regulators of histone methylation primarily affect HCC resistance to chemotherapy drugs and cancer stem cell pluripotency through the modification of methyltransferases and demethylases. A number of studies have indicated that, during protein methylation modification arginine, methyltransferases PRMTs may be suitable as targets for the development of drugs to treat cancers by inhibiting epigenetic methylation. The concept has been validated in other cancer studies. In cellular experiments, inhibitors of type I-PRMTs were demonstrated to have anti-tumour effects in a melanoma xenograft model, indicating that the compounds warrant further investigation as a potential anti-cancer agent.[134] Moreover, targeting of type I-PRMT has the potential to enhance the efficacy of immunotherapy in patients with triple-negative breast cancer (TNBC).[135] Further studies are required to elucidate the specific role of PRMT in HCC and to identify effective targeted therapies.

Discussion

The occurrence of dynamic methylation overlap is observed throughout the development of HCC. The three methylation modes (DNA, RNA, and protein methylation) cross-talk with each other and are closely related to chromosome remodelling and gene expression, which likely plays a pivotal role in tumor development and transformation. Aberrant DNA methylation is a primary alteration that drives tumorigenesis, while RNA and protein methylation predominantly influence tumor progression through transcription and translation. All three methylation modifications can affect tumour progression. Epigenetic methylation modifications can act via pathways such as Wnt/beta-catenin, PI3K/Akt/mTOR and ATK/ERK/c-MYC/CDK. Furthermore, we have provided a summary of the target genes that are crucial for the methylation modification process, as well as the relationship between the target genes and the signalling pathways in Figure 1.

In recent years, studies have shed light on the significant role of methylation in the HCC tumor microenvironment. Methylation has been implicated in HCC development and drug resistance, however, its precise effects on HCC remain elusive as well as require further investigation. Emerging evidences suggest a potential connection between methylation, ferroptosis, and autophagy.[136] Since modifications of the m6A RNA can regulate autophagy to modulate HCC growth or development.[137] Recent discovery that H4 forms doubly modified acetylmethyllysine, which has all the characteristics of post-translational modification. It has great significance for further study on the pathogenesis of cancer. Notably, Investigating the interference of methylation at the HCC locus in autophagy and exploring the potential of methylation inhibitors or inducers to overcome drug resistance in HCC are areas that warrant further exploration. However, given the significant role of methylation in genetic information, it holds promise as a potential tool for the early detection of HCC and may serve as a new target for improving drug resistance in HCC cells and promoting apoptosis. Exploring the possibility of incorporating methylation indicators into individualized testing parameters requires further investigation. Epigenetic liquid biopsies, which detect abnormal immune environments by examining methylation, fragmentation, and histone labeling patterns of decellularized DNA in blood,[138] offer a promising avenue for investigation. Moreover, several experimental studies have demonstrated that targeted inhibition of aberrant DNA methylation in HCC may affect tumorigenesis. Modifying methylation-associated enzymes and their binding proteins to target the transcription-translation axis presents a promising therapeutic strategy. In brief, the prospect of methylation in HCC research, particularly the feasibility of using agonists or inhibitors targeting enzymes and binding proteins involved in methylation processes to treat HCC patients, requires further investigation.

In conclusion, this review confirms that methylated genes have the potential to be biomarkers for the diagnosis and prognosis of HCC and demonstrates that different types of methylation modifications affect the cancer cell phenotype and

the development of HCC through the regulation of signalling pathways by different enzymes and binding proteins. However, further research is needed in HCC targeted therapy in the future.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests in this work.

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