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Review

5-Hydroxymethylcytosine and disease

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ABSTRACT

Epigenetics is the study of inherited changes in phenotype or gene expression that do not alter DNA sequence. Recently, scientists have focused their attention on 5-hydroxymethylcytosine (5hmC), a newly discovered epigenetic marker, also known as sixth DNA base of the genome. In mammals, this novel epigenetic marker is derived from 5-methylcytosine (5mC) in a process catalyzed by ten-eleven translocation (TET) enzymes. Although 5hmC has only been subjected to study for a short while, a great deal of data has been accumulated regarding its generation, distribution, demethylation, function, and disease implications. All this information suggested that 5hmC acts not only as an intermediate in the DNA demethylation process but also as an independent epigenetic marker, playing an important role in the regulation of gene expression. This review focuses on recent progress in the study of the relationship between 5hmC and human diseases, such as cancer and Rett syndrome (RTT).

Contents

Abbreviations: 2-HG, 2-hydroxyglutarate; 5mC, 5-methylcytosine; 5hmC, 5-hydroxymethylcytosine; 5fC, 5-formylcytosine; 5caC, 5-carboxylcytosine; a-KG, a-ketoglutarate; AD, Alzheimer's disease; A2AR, adenosine A2A receptor; AML, acute myeloid leukemia; AAs, anaplastic astrocytomas; BCN, benign compound nevi; C5, 5-position; CMD, cutaneous metastatic disease; cHILIC-ESI-qTOF-MS/MS, capillary hydrophilic-interaction liquid chromatography/quadrupole TOF mass spectrometry; CMML, chronic myelomonocytic leukemia; CML, chronic myeloid leukemia; CA, cornus ammonis; CER, cerebellum; DhMRs, differential 5-hydroxymethylated regions; DCN, dysplastic compound nevi; DEN, diethylnitrosamine; DG, dentate gyrus; DGo, dentate gyrus outer layer; DGi, dentate gyrus inner layer; ESCs, embryonic stem cells; E17, embryonic 17; GISTs, gastrointestinal stromal tumors; GBMs, glioblastomas; hAPP, human amyloid precursor protein; HD, Huntington's disease; HTT, huntingtin; hMeDIP-seq, hydroxymethylated DNA immunoprecipitation sequencing; HPG, hippocampus/parahippocampal gyrus; ICT, isocitrate; IDH1/2, isocitrate dehydrogenase1/2; IR, ischemia-reperfusion; LC–MS/MS, liquid chromatography–electrospray ionization tandem mass spectrometric; LC–MS, liquid chromatography–mass spectrometry; LAD, late-stage AD; MeCP2, methyl-CpG-binding protein 2; MeDIP-seq, methylated DNA immunoprecipitation sequencing; MLL, myelomonocytic leukemia; MDS, myelodysplastic syndromes; MPNs, myeloproliferative neoplasms; NURD, nucleosome remodeling and histone deacetylation; NE, neuroepithelial; NeuN, neuron-specific nuclear protein; oxBS-Seq, oxidative bisulfite sequencing; OS, overall survival; P7, postnatal day 7; PCAD, preclinical AD; RTT, Rett syndrome; SSM, superficial spreading melanomas; SDH, succinate dehydrogenase; TET, ten-eleven translocation; TDG, thymine DNA glycosylase; TAB-Seq, tet-assisted bisulfite sequencing.

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1. Introduction

DNA methylation at the 5 position of cytosine in the mammalian genome is a key epigenetic event, which the biochemical process is catalyzed by DNA methyltransferases (DNMTs) usually at the CpG dinucleotides of the cytosine ring [1]. 5mC plays pivotal roles in transcriptional silencing of specific genes and repetitive elements [2], genomic imprinting [3], inactivation of the X-chromosome [4], cell differentiation [5], and tumorigenesis [6].

For a long time, 5mC was known as a static and stable epigenetic marker of DNA. But it was not until 2009 that two independent groups reported that 5hmC was derived from 5mC, which was also found to be ubiquitous in mammalian DNA, indicating that DNA methylation is not static but dynamic and that 5hmC may serve as an intermediate in the demethylation of active DNA [7,8]. This finding has profoundly enriched understanding of intricate epigenetics. It has also stimulated scientific interest to uncover its mysterious veils in gene regulation.

Numerous studies have shown that many proteins are involved in DNA active demethylation, including Gadd45a [9], Elp3/KAT9 [10], AID/APOBEC [11–14], MBD4 [15,16], virion protein16 (Vp16) [17], SMUG1 [18], TGF-β [19], and FGF [20]. TETs and thymine DNA glycosylase-mediated (TDG-mediated) active demethylation are considered a classic pathway [21–31]. Human TET proteins contain three components, TET1, TET2, and TET3 [8,32]. These belong to the a-ketoglutarate-dependent (a-KG) and Fe(II)-dependent dioxygenase superfamily. 5mC can be catalyzed to 5hmC by TETs and further oxidized to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) [33,34]. The markers 5fC and 5caC can be converted back to unmodified cytosine via TDG and base excision repair (Fig. 1) [35].

Recently, some researches have shown that IDH1/2 can catalyze isocitrate (ICT) to a-KG, and that they are involved in the regulation of TETs and 5hmC, but mutant IDH1/2 has been found to catalyze ICT to 2-hydroxyglutarate (2-HG), which acts as a competitive inhibitor of a-KG. It can also inhibit the conversion of 5mC to 5hmC by TETs. In this way, IDH1/2 may play an important role in the regulation of 5hmC [36–38] (Fig. 2).

Moreover, the levels of 5hmC varied in different organs. The content of 5hmC is highest in brain, following the rectum, liver and colon. However, the heart, breast and placenta are very low. And they also reported that 5mC are expressed in all tissues; the contents of 5mC were ranged from 0.6% to 1.5% as shown in Table 1 [39].

Studies have also confirmed that 5hmC is also deeply involved in normal functions, such as brain development in mice [40], neuronal differentiation in human brains, and self-renewal in embryonic stem cells (ESCs) of mice [32]. The 5hmC-specific binding proteins Mbd3/NURD and MeCP2 have been identified in ESCs and in the nervous system, respectively [41,42]. This suggests that 5hmC may serve not only as an intermediate in demethylation but also as a novel independent epigenetic marker and regulator, and its dysregulation may cause some diseases, such as cancer and Rett syndrome (RTT) [43–46].

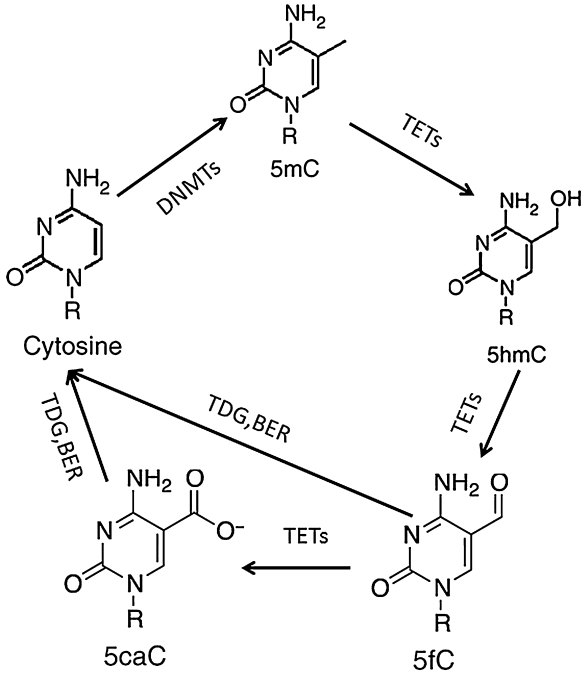

Fig. 1. Derivation and position of 5-hydroxymethylcytosine. Cytosine can be converted to 5mC by DNMTs. 5mC can be converted to 5hmC by TETs and further oxidized to 5fC and 5caC. 5fC and 5caC can be converted back to unmodified cytosine via TDG and base excision repair.

2. 5hmC and diseases

A great number of recent studies have been performed on the function of 5hmC. Data have shown that 5hmC may have functions in the cytoskeleton, ion transport, transcription, cell adhesion, cell death, development, differentiation, maturation, chromatin structure, splicing, self-renewal, and myelopoiesis [7,8,32,40,42,43,46–54]. When the distribution and concentration of 5hmC change, disease occurs (Table 2).

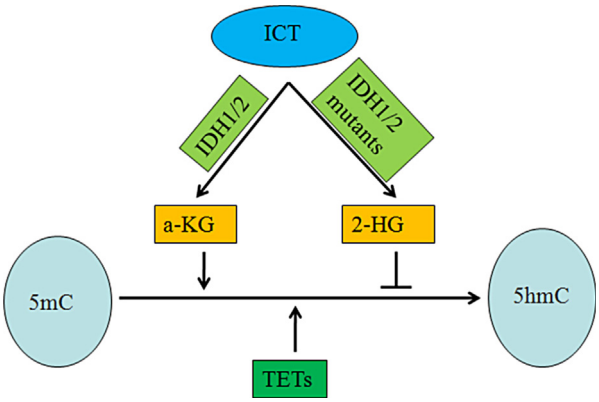

Fig. 2. The relationship between TETs, IDH1/2 and 5hmC. ICT can be catalyzed by IDH1/2 and its mutants, and transformed into a-KG and 2-HG, respectively. Acting as a cofactor of TETs, a-KG can facilitate TETs to catalyze 5mC into 5hmC, whereas competitive inhibitor of a-KG, 2-HG can disturb the process of hydroxymethylation.