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Detection of mismatched 5-hydroxymethyluracil in DNA by selective chemical labeling

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ABSTRACT

How DNA demethylation is achieved in mammals is still under extensive investigation. One proposed mechanism is deamination of 5-hydroxymethylcytosine to form 5-hydroxymethyluracil (5hmU), followed by base excision repair to replace the mismatched 5hmU with cytosine. In this process, 5hmU:G mispair serves as a key intermediate and its localization and distribution in mammalian genome could be important information to investigate the proposed pathway. Here we describe a selective labeling method to map mismatched 5hmU. After converting other cytosine modifications to 5-carboxylcytosines, a biotin tag is installed onto mismatched 5hmU through β-glucosyltransferase-catalyzed glucosylation and click chemistry. The enriched 5hmU-containing DNA fragments can be subject to subsequent sequencing to reveal the distribution of 5hmU:G mispair with base-resolution information acquired.

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1. Introduction

5-Methylcytosine (5mC) is an important epigenetic mark in mammalian cells and is regarded as the fifth base besides A, T, C and G. 5mC has been known to impact various biological functions such as genomic imprinting, X chromosome inactivation and cancer development [1,2]. Although 5mC appears to be a relatively stable modification, there are clear observations of demethylation in both zygotes and somatic cells, ranging from genome-wide to a few loci [3]. However, it is still obscure how demethylation is initiated with multiple pathways proposed [3]. Recently, the discovery of oxidative derivatives of 5mC in mammalian cells opens up new possibilities for demethylation mechanisms (Fig. 1) [4–7]. Pathway 1 is thymine DNA glycosylase (TDG)-mediated base excision repair (BER). Both 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC), oxidized products of 5mC, can be recognized by TDG and converted back to unmodified cytosine in high efficiency [6,8,9]. Pathway 2 is direct decarboxylation of 5caC. Although no decarboxylase has been identified, decarboxylation activity in mouse embryonic stem (mES) cell lysate has been suggested [10]. Pathway 3 is deamination of 5hmC followed by BER. AID (activation-induced deaminase)/APOBEC (apolipoprotein B mRNA-editing enzyme complex) family proteins, which catalyze the deamination of cytosine to uracil, may also work on 5hmC to produce 5hmU:G mispair that can be excised by glycosylases and repaired through BER [11,12]. It has been reported that the amount of 5hmU is positively correlated with the expression level of Tet1 but negatively correlated with the expression level of AID *in vivo*, suggesting AID/APOBEC may contribute to demethylation [12]. However, recent biochemistry study showed that purified AID/APOBECs exhibit very low deamination activity on 5hmC, raising questions about the feasibility of this pathway [13].

To further explore the plausibility of deamination-involved demethylation pathway, it will be important to gain the knowledge about the localization of mismatched 5hmU, derived directly from 5hmC deamination. Therefore, an efficient and robust labeling approach is required. The structural similarities between 5hmC and 5hmU make it possible to apply certain 5hmC labeling and profiling methods to 5hmU. β-Glucosyltransferase (βGT) from T4 bacteriophage is known to catalyze the glucosylation reaction on the hydroxyl group of 5hmC. We found that βGT can also work on mismatched 5hmU:G but not matched 5hmU:A, which prompted us to design the following strategy for chemical labeling of mismatched 5hmU (Fig. 2). First, recombinant mouse Tet1 is utilized to oxidize all 5mC and 5hmC in genomic DNA to 5caC. Then βGT is applied to install a modified N_3-glucose onto the hydroxyl

Abbreviations: 5mC, 5-methylcytosine; 5hmC, 5-hydroxymethylcytosine; 5fC, 5-formylcytosine; 5caC, 5-carboxylcytosine; 5hmU, 5-hydroxymethyluracil; BER, base excision repair; TET, Ten-eleven translocation; TDG, thymine DNA glycosylase; βGT, β-glucosyltransferase; AID, activation-induced deaminase; APOBEC, apolipoprotein B mRNA-editing enzyme complex; UDP-Glc, uridine diphosphoglucose; mES, mouse embryonic stem.

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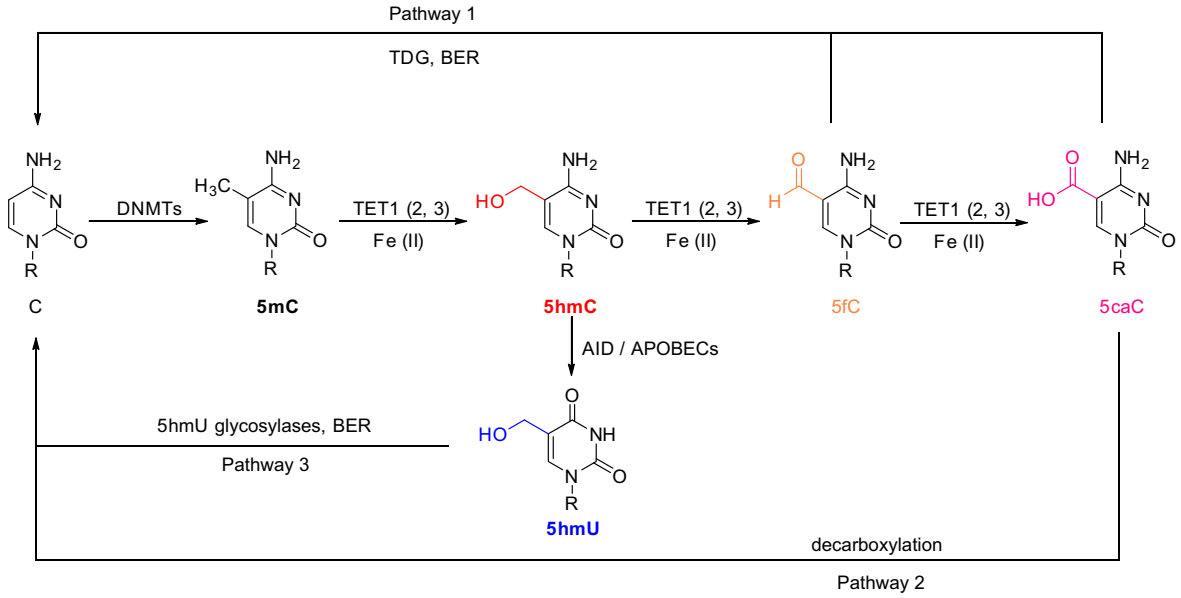

Fig. 1. Proposed 5mC demethylation pathways in mammals. Pathway 1, cleavage of 5fC and 5caC by TDG followed by BER; Pathway 2, direct decarboxylation of 5caC; Pathway 3, deamination of 5hmC followed by BER.

group of mismatched 5hmU followed by incorporation of disulfide biotin linker through click chemistry. After capture of the mismatched-5hmU-containing fragments with streptavidin-coupled beads, the bound DNA fragments can be readily released by simple DTT cleavage of the disulfide bond. The enriched fragments can be applied to deep sequencing to map the distribution of mismatched 5hmU. The precise position of the mismatched 5hmU may be determined by analyzing C-to-T mutation around the identified peaks.

1.1. Mismatched 5hmU chemical labeling with N_3-glucose and biotin

βGT transfers the glucose moiety from uridine diphosphoglucose (UDP-Glc) to 5hmC on double-stranded DNA [14,15]. Previously, we have demonstrated that βGT can utilize modified UDP-6-N_3-Glc as a cofactor with only slight decrease in reaction rate for 5hmC substrate [16]. However, there is not much study about the activity of βGT on 5hmU. To test whether βGT works on 5hmU in a similar way, we attempted the glucosylation reaction on a model 9mer-11mer duplex DNA containing one mismatched 5hmU (5hmU:G) or matched 5hmU (5hmU:A) site, and monitored the products by MALDI-TOF/TOF. In the presence of 2 μM βGT and 200 μM UDP-6-N_3-Glc, only mismatched 5hmU could be glucosylated to form N_3-5gmU while matched 5hmU stayed untouched (Fig. 3 and Fig. S1A). The yield of glucosylation reaction on mismatched 5hmU is over 90%, which was confirmed by HPLC (Fig. S2). The resulting N_3-5gmU could be further modified to add a biotin tag by reacting with the disulfide-containing biotin linker through copper-free click chemistry to yield biotin-S-S-N_3-5gmU.

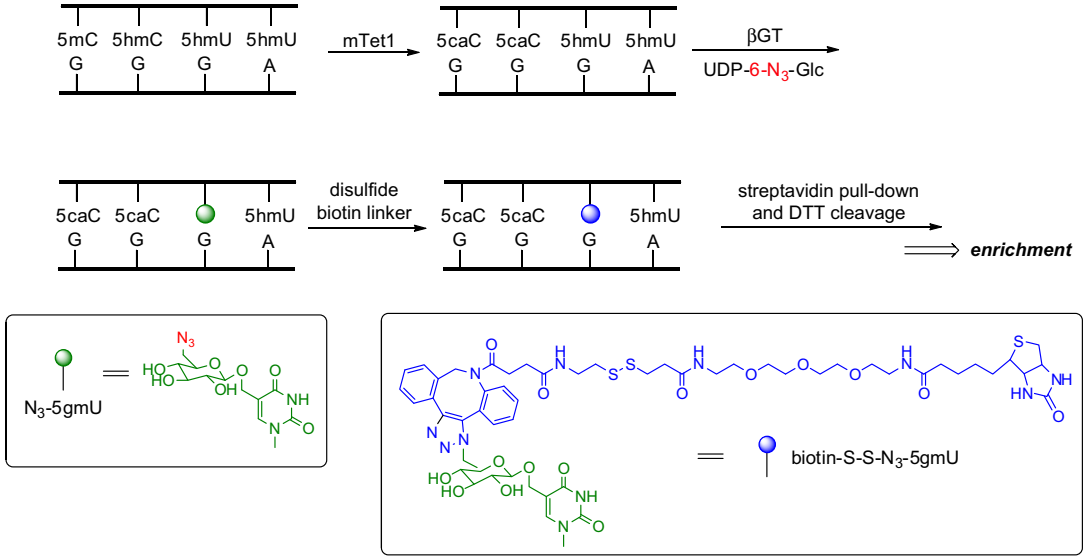

Fig. 2. Overview of the chemical labeling and enrichment strategy for mismatched 5hmU. Genomic DNA is first treated with recombinant mTet1 to convert all 5mC and 5hmC to 5caC. Then βGT is utilized to selectively label mismatched 5hmU with N_3-glucose. After adding the biotin tag through click chemistry, the mismatched 5hmU-containing DNA fragments are enriched by streptavidin-coupled beads.

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