Mutant cytosine modifying enzymes to enhance epigenetic or base-editing on genomic DNA

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Technology description

Mutant TET enzymes that stall the oxidation reaction of 5-methylcytosine (5mC) at 5-hydroxymethylcytosine (5hmC), and AID/APOBEC enzymes with hyperactive deamination activity.

Problem

Epigenetic modifications to DNA, such as cytosine methylation, play critical roles in modulating gene expression without changing coding sequences. Changes or editing of bases is also critical in immune defense. Localizing of targeting modifications or mutations is important to understanding or manipulating physiological and pathological processes.

Solution

Dr. Kohli and his colleagues at University of Pennsylvania developed mutant cytosine modifying enzymes; specifically, AID (activation-induced cytidine deaminase), APOBEC (apolipoprotein B editing complex), and TET (ten eleven translocation) enzymes with improved functions. Hyperactive AID or hyperactive APOBEC proteins can be used in APOBEC-coupled epigenetic sequencing (ACE-Seq) or other epigenetic sequencing methods to distinguish cytosine from modified cytosine bases. TET mutants are useful for stalling oxidation reactions at hmC and/or introducing hmC modifications into specific sites in the genome. In addition to epigenetic applications, these mutant enzymes can be valuable in genome or epigenome editing in combination with CRISPR or other tools for targeting.

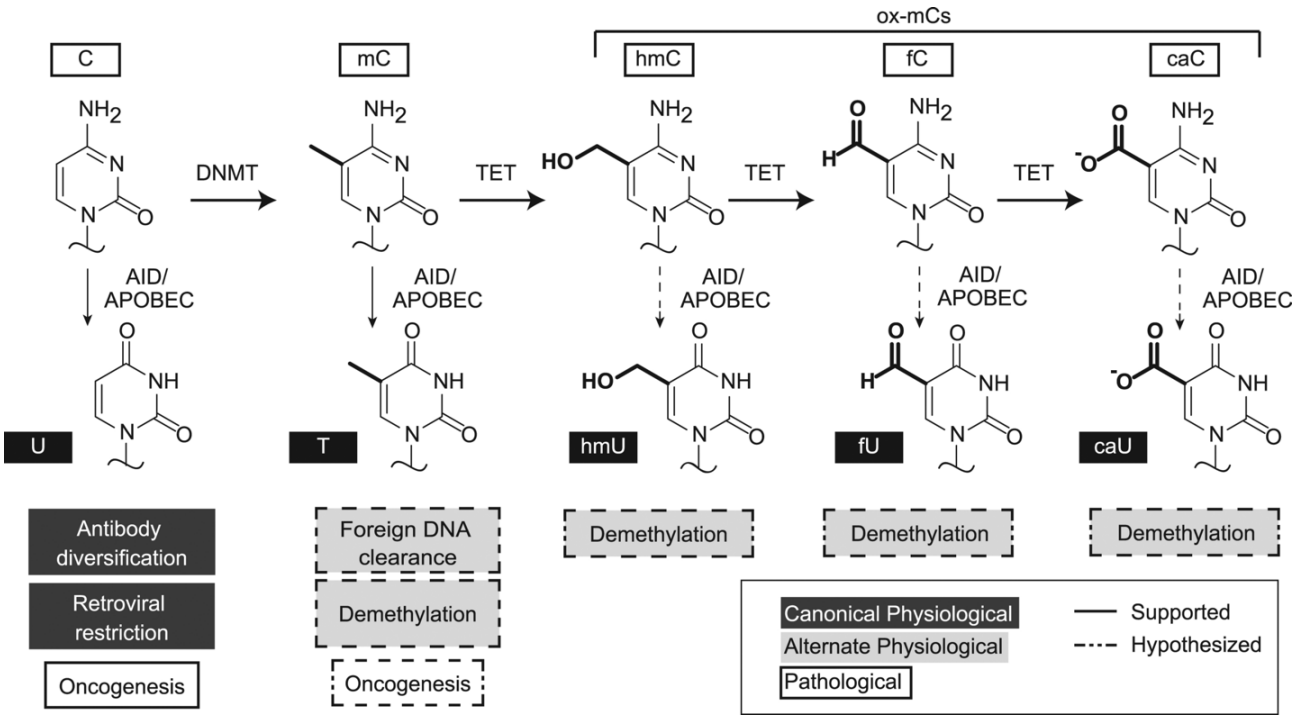

Figure.TET enzymes sequentially oxidize 5-methylcytosine (mC) into three additional bases: 5-hydroxymethylcytosine (hmC), 5-formylcytosine (fC), and 5-carboxylcytosine (caC). AID/APOBEC enzymes deaminate cytidine bases to uridine analogs and preferentially act on ssDNA. The DNA modifications by these enzymes are critical for many physiological actions.

Figure from Schutsky et al. Nucleic Acids Res, 2017, 45 (13) – 7655.

Reference Media

Gajula et al. Nucleic Acids Res, 2014, 42 (15) – 9964

Liu et al. Nat Chem Biol, 2017, 13 (2) – 181

Application area

- Sequencing
- Gene editing
- Epigenome editing

Advantages

- More controllable enzymes.
- Increased base-editing efficiency and precision.

Institution

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