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Rationale Design of Inhibitors based on Crystal Structures of ssDNA Bound to APOBEC

IP Status: Issued US Patent; **Issued Patent No.** 11,192,929

Highly effective inhibitor of solar UV-induced skin cancer

DNA Base Editing Enzymes with High Specificity and Efficiency

A new method will help achieve specific DNA editing events with fewer off-target issues by using modified Cas9-APOBEC fusion polypeptides. Crystal structures for ssDNA bound APOBEC3A and APOBEC3B can be used for rational design of inhibitors to impede the evolvability of viruses and tumors and for development of APOBEC-mediated base editing reporter (AMBER) systems.

Better Tool for DNA Editing In Vivo

Better tools are needed for DNA editing in vivo. The original APOBEC1-Cas9 base editing complexes have wide “editing windows”, which are nearly as big as the >20 nucleotide single-stranded DNA region displaced by annealing of the guide-RNA that directs the editing complex. The high-resolution structural information for APOBEC3A and APOBEC3B (catalytic domain) enzymes in complex with relevant single-stranded DNA substrates provide atomic-level explanations for their strong specificity for 5′-TC-3′ dinucleotide sequences within longer single-stranded DNA substrates. They also provide strong structural rationale that has already enabled the local specificity of these enzymes to be changed to 5′-CC-3′. Structural information for these extremely efficient enzymes makes it possible to tune the enzyme to preferentially edit 5′-AC-3′ and 5′-GC-3′ dinucleotide targets. Thus, these enzymes expand the DNA editing toolkit to be able to selectively target DNA cytosine bases in any dinucleotide context. In addition, unlike the normal CRISPR system, base editing requires neither double-strand DNA cleavage nor a DNA donor template.

Complementary Technologies

APOBEC3A- and APOBEC3B (catalytic domain)-Cas9 base editing complexes may be targeted with an appropriate guide RNA to virtually any DNA target. They may also be used in combination with a fluorescent reporter for DNA editing (i.e., the APOBEC- and Cas9-mediated editing (ACE) reporter system).

Phase of Development

- Proof of concept. Further refinement and optimization on going.

Benefits

- Achieves specific DNA editing events with fewer off-target issues
- Enables additional fine-tuning of editing complexes by structure-guided mutagenesis and/or high-throughput screens
- Enables screens for chemical modifiers (inhibitors or activators) of ssDNA editing

Technology ID
20170124

Category
Life Sciences/Human Health
Life Sciences/Research Tools
Life Sciences/Therapeutics
Agriculture & Veterinary/Ag Biotechnology
Agriculture & Veterinary/Veterinary Medicine

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Features

- Relies upon high-resolution crystal structure information for ssDNA bound APOBEC3A and APOBEC3B
- May be used alone or in combination with a fluorescent reporter for DNA editing (ACE)

Applications

- Gene editing in living cells
- Rational design of inhibitors to impede the evolvability of viruses and tumors
- Use alone or in combination with a fluorescent reporter for DNA editing (i.e., the APOBEC- and Cas9-mediated editing (ACE) reporter system)

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Publications

Structural basis for targeted DNA cytosine deamination and mutagenesis by APOBEC3A and APOBEC3B

Nature Structural & Molecular Biology , Volume 24, pages 131–139 (2017)

A fluorescent reporter for quantification and enrichment of DNA editing by APOBEC–Cas9 or cleavage by Cas9 in living cells

Nucleic Acids Research, Volume 46, Issue 14, 21 August 2018, Pages e84

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