Novel Artificial Endonuclease Sandwiched with Two Zinc-finger Proteins

This invention enables us to cleave genomic DNA at a specific site. Therefore, the invention leads to engineering of genome sequences/genetic information such as gene knockout and gene targeting via homologous recombination or nonhomologous end joining. For example, using this technology, we may be able to generate engineered agricultural crops and novel disease model animals.

Technology Area
Medicine, Pharma & Biotechnology

Keywords
Artificial Endonuclease
Zinc-Finger Protein
Genome Engineering
Gene Knockout
Gene Targeting

Researchers
Takashi SERA

Summary
Zinc-finger nucleases (ZFNs) are a powerful tool for manipulation of genomic DNA. However, ZFNs still have some defects such as nontargeted cleavage due to the homodimerization and no/low turnover DNA cleavage. To refine ZFN technology, we developed a novel DNA-cleaving domain, a single-chain FokI dimer (scFokI) and sandwiched the scFokI with two artificial zinc-finger proteins (AZPs). The resulting sandwiched ZFNs cleaved double-stranded DNA (dsDNA) at a single nucleotide position of target DNA, in which two AZP-binding sites were connected with a 6-bp spacer, with multiple turnovers like native restriction endonucleases. The novel artificial endonucleases will be used to engineer genome sequences/genetic information, leading to generation of useful organisms such as new agricultural crops and disease animal models.
The sandwiched ZFN cleaved dsDNA at a single site. The reaction mixtures of 5'-end-labeled 200-bp dsDNA targets cleaved by the sandwiched ZFN were separated on a 6% denaturing gel. The sandwiched ZFN produced a 2-bp sticky end. Black bars next to the C + T marker lane (M) indicate the binding sites of two AZPs used. Boxes indicate DNA sequences of the binding sites of these AZPs, and the main cleavage sites are donated with black arrows. S, DNA substrate; P, DNA cleavage products.
Figure 2

The sandwiched ZFN cleaved dsDNA with multiple turnovers. A target plasmid was incubated with a FokI variant at 37℃ for 8 h (lanes 1 and 2) or 17 h (lanes 3 and 4) and then digested with XmnI. Under the conditions, our sandwiched ZFN demonstrated much greater DNA-cleaving activity than that of conventional ZFN. Amounts of DNA used are shown relative to FokI variants (final concentration: 5 nM). S, a 2.9-kb DNA substrate; P1, a 0.9-kb cleavage product; P2, a 2.0-kb cleavage product.

Additional Information

Related papers/documents/links:

1. WO2007/102618, 'Nucleic Acid Cleaving Agent' (Inventor: Takashi Sera*. Publication date: September 13, 2008)
2. Tomoaki Mori, Ikuko Kagatsume, Kazuki Shinomiya, Yasuhiro Aoyama, Takashi Sera*, 'Sandwiched zinc-finger nucleases harboring a single-chain FokI dimmer as a DNA-cleavage domain', Biochemical and Biophysical Research Communications, 390 (2009), 694-697
3. Takashi Mino, Yasuhiro Aoyama, Takashi Sera*, 'Efficient double-stranded DNA cleavage by artificial zinc-finger nucleases composed of one zinc-finger protein and a single-chain FokI dimmer', Journal of Biotechnology, 140 (2009), 156-161
5. Takashi Sera* and Carla Uranga, 'Rational Design of Artificial Zinc-Finger Proteins' (2012) (c) Copyright Kyoto University. All rights reserved.