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DNA Modification Enzymes Utilizing Sequence-Specificity of Zinc Finger Domains

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Artificial zinc finger proteins (ZFPs) consist of Cys_2-His_2-type modules composed of about 30 amino acids with a ββα structure that coordinates a zinc ion. ZFPs that recognize specific DNA target sequences can substitute for the binding domains of enzymes that act on DNA to create designer enzymes, such as nucleases, recombinases [1], and methylases [2], with programmable sequence specificity. Genome editing and modification by the enzymes could be applied for many fields of basic research and medicine. Some zinc finger nucleases (ZFN) are currently in human clinical trials that aim at therapeutic gene editing. In this presentation, ZFN pairs targeting promoter region of human telomerase reverse transcriptase (hTERT) were designed and constructed. Expressed zinc fingers were purified in vitro and the DNA binding properties were evaluated. Highly active zinc fingers were utilized for ZFN binding domain. The DNA cleavage of ZFNs was performed in vitro. Furthermore, the digestion of promoter region in the endogenous sequences was successfully confirmed. The telomerase activity in the cell treated by ZFN was reduced suggesting the digestion by ZFN affects to the expression of hTERT. There are few reports about the strategy with ZFN targeting the promoter region of the target gene. The present results give a new insight into the gene targeting and editing by sequence-specific nucleases. In addition, our current accomplishments on zinc finger enzymes, such as the zinc finger recombinase (ZFR) design and ZFN targeting replication sites of EB virus, will be presented.

[1] Nomura, W., Masuda, A., Ohba, K., Urabe, A., Ito, N., Ryo, A., Yamamoto, N., and Tamamura, H., Effects of DNA binding of zinc finger and linkers for domain fusion on catalytic activity of sequence-specific chimeric recombinases determined by a facile fluorescent system, *Biochemistry*, 51:1510-1517, 2012.
[2] Nomura, W., and Barbas, C. F., III, In vivo site-specific DNA methylation with a designed sequence-enabled DNA methylase, *J. Am. Chem. Soc.*, 129:8676-8677, 2007.