Engineering Newly Discovered Nucleases to Modify Human Genes

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The technical challenges associated with gene therapy have restrained this otherwise powerful therapeutic approach (e.g., delivery of gene modifying reagents to cells of interest while ensuring target gene specificity). However, postdoctoral fellow Dr. Ryo Takeuchi and colleagues in the Basic Sciences Division recently demonstrated an approach that makes site-specific gene modification in human cells feasible.

Homing endonucleases (HEs), zinc finger nucleases (ZFNs) and transcription activator-like (TAL) effector nucleases, are all highly specific DNA recognition and cleavage enzymes that are currently being explored as gene modification reagents. The double-strand breaks generated by these enzymes induce homologous recombination or nonhomologous end joining (NHEJ) repair responses, depending on whether a DNA template with homology to the break site is available. Takeuchi *et al.* identified 211 putative LAGLIDADG homing endonucleases (LHE) using structure-based alignments, and confirmed targeted DNA cleavage activity for six out of 11 recombinantly expressed members of the I-OnuI subfamily, which includes the recently discovered I-OnuI and I-LtrI LHEs. To understand how I-OnuI recognizes its target sequence and thus modify this specificity, they solved the crystal structure of I-OnuI bound to its 22 base pair DNA target and then determined which nucleotide-residue interactions were tolerant to substitution. They used directed evolution to alter the specificity of I-OnuI to that of a closely related DNA target, monoamine oxidase B (MAO-B), which is a candidate therapeutic target for a number of neurodegenerative disorders, including Parkinson's disease. The resulting variant, E2 I-OnuI, contains only nine amino acid substitutions and efficiently alters its MAO-B target site in human HEK 293T cells. Although Takeuchi *et al.* observe some E2 I-OnuI activity at off-target DNA sequences with homology to the MAO-B target, their general approach demonstrates that directed evolution of LHEs with diverse wild-type target sequences holds promise as a means to generate a number of gene-specific modification reagents.

Takeuchi R, Lambert AR, Mak ANS, Jacoby K, Dickson RJ, Gloor GB, Scharenberg AM, Edgell DR, Stoddard BL. 2011. Tapping natural reservoirs of homing endonucleases for targeted gene modification. *Proc Natl Acad Sci USA* 108:13077-82.