

An enhanced CRISPR tool for treating chronic myelogenous leukemia

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The CRISPR/Cas system is a highly potent tool which has revolutionized genome engineering and regulation of gene transcription in various cells and organisms. This gene-editing tool consists of a guide RNA (gRNA) and Cas9 endonuclease. Cas9 catalyzes the formation of double-strand DNA breaks, which are then repaired by different cell mechanisms. Error-prone Non-homologous end joining occurs, resulting in random indel (insertion-deletion) mutations, which can lead to functional gene inactivation by either frameshift or deletions. To achieve greater indel mutations, CRISPR system can be coexpressed in cells with DNA exonucleases, which cause increased recessions of DNA following DNA breaks. We show that joint action of the CRISPR system with different exonucleases significantly increases the percentage of indel mutations at various targeted genes. Of the different exonucleases tested, the E.coli-derived exonuclease III (EXOIII) exhibited the best performance in terms of indel formation. To further improve the rate of indel mutations, Cas9 and EXOIII were brought into the proximity via coiled-coil forming heterodimeric peptides (CCExo). This resulted in increased indel formation compared to the classical CRISPR/Cas system as well as more efficient than cointroduction of non-interacting and genetically fused Cas9-EXOIII. We performed a case study for the use of the CRISPR-EXO system as a potential anti-cancer therapeutic tool. The Philadelphia chromosome, which occurs in leukemic cancer cells, is the result of characteristic the reciprocal genome translocation t(9:22) and is responsible for higher proliferation of tumorous cells. Using the CCExo system, we achieved a higher degree of indel mutations at the translocation site, which resulted in greater killing of cancer cells, thus providing a useful potential anti-cancer therapeutic tool.