The the central component of the type ii

The prokaryotic type II clustered regularly interspaced shortpalindromic repeats (CRISPR)-Cas9 system is rapidly revolutionizing the fieldof genetic engineering, allowing researchers to alter the genomes of a largevariety of organisms with relative ease.

Experimental approaches based on thisversatile technology have the potential to transform the field of cancergenetics 1. This Cas9 endonuclease is the central component of the TypeII CRISPR/Cas system, a prokaryotic adaptive restriction system againstinvading nucleic acids, such as those originating from bacteriophages andplasmids. Recently, this RNA-directed DNA endonuclease has been harnessed totarget DNA sequences of interest. Through guidance of a 20nucleotide RNA (gRNA), CRISPR-Cas9 finds and cuts target protospacer DNAprecisely 3 base pairs upstream of a PAM (Protospacer Adjacent Motif) 2. Cas9 is as an important tool to not only edit thegenomes of a number of different prokaryotic and eukaryotic species, but alsoas an efficient system for site-specific transcriptional repression oractivation 3 it couldalso be used to modify any genomic sequences, thereby providing a specific, simple, easy, and cost effective means of genome wide gene editing, analogous to thesearch function in modern word processors, Cas9 can be guided to specificlocations within complex genomes by a short RNA search string. Using thissystem, DNA sequences within the endogenous genome and their functional outputsare now easily edited or modulated in virtually any organism of choice. Cas9-mediated genetic perturbation is simple and scalable, empoweringresearchers to elucidate the functional organization of the genome at thesystems level and establish causal linkages between genetic variations andbiological phenotypes 4 it is

also aflexible, RNA-guided DNA recognition platform, which enables precise, scalable androbust RNA-guided transcription regulation 5, in contrast tothis, RNA-mediated interference (RNAi), which uses small interfering RNAs(siRNAs) or short hairpin RNAs (shRNAs), has also been used for sequence-specific gene suppression in eukaryoticorganisms 6 but it is non-specificand inefficient 7.

Genome engineering via the RNA-guided CRISPR-Cas9system provides a novel methodology, allowing induction of genomicmodifications under the endogenous gene promoters 8. During the last few years, the clustered regularlyinterspaced short palindromic repeats (CRISPR) and the associated Cas9nucleases (CRISPR-Cas9) have revolutionized the options for targeted genomeediting 9. These programmable RNA-guidedendonucleases(RGENs)comprise two RNA elements, CRISPR RNA (cRNA) and its transactivating RNA(tracRNA), which can be fused together and used to induce a targeteddouble-strand break (DSB). Providing a corresponding DNA template, any specificgene sequence can be introduced via homologous recombination (HR) 10. CRISPR/Cas is a microbial adaptive immune system that usesRNA-guided nucleases to cleave foreign genetic elements, uses a single-guide RNAto target the Cas9 nuclease to a specific genomic sequence. Cas9 inducesdouble-stranded DNA breaks which are repaired either by imperfectnon-homologous end joining to generate insertions or deletions (indels) or, ifa repair template is provided, by homology-directed repair.

Due to itsspecificity, simplicity and versatility, the CRISPR/Cas9 system has recentlyemerged as a powerful tool for genome engineering in various

species 11. Recent reports on CRISPRscreenson several cancercell lines have demonstrated their power to cure cancer 12. A human malignancy in urgent need of additional therapiesis acute myeloid leukemia (AML), a devastating disorder with a long-termsurvival rate of less than 30% (Ferrara andSchiffer, 2013). Steady progress in deciphering its molecularpathogenesis has been made over the last few decades with a dramaticacceleration in recent years, particularly as a consequence of advances incancer genomics (Cancer Genome Atlas Research Network, 2013; Welch et al.

, 2012).