CRISPR-Cas9 Mediated Gene Editing: A Revolution in Genome Engineering

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Commentary

CRISPR-Cas technology has taken over the laboratories around the world as a revolution. This genome engineering technology is powerful in that it provides a robust method of systematically analyzing gene functions, disease target screening and validation, gene knockout studies for factors contributing to tumor progression, genome imaging, screening etc. Its vast applications are only emerging and in the future, hold a great promise towards better understanding of disease progression and accelerate the cure.

CRISPR-Cas9 systems emerged from bacteria and archaea where this mechanism is used for conferring adaptive immunity against bacteriophages and plasmids [1,2]. CRISPR stands for “Clustered regularly interspersed short palindromic repeats”. Its associated protein Cas9 is an endonuclease that uses a RNA duplex of tracr RNA: cr RNA (trans-activating cr RNA and CRISPR targeting RNA) as a guide to form base pairs with the target DNA sequences thus allowing Cas9 to make a site-specific Double Strand Breaks (DSB). This dual tracr RNA: cr RNA can be engineered as a single guide RNA (sgRNA) sequence that has a 20nt at the 5’ end of the sgRNA which is required for determining the target site and a duplex RNA structure at the 3’ end that binds to the Cas9. This design allows changes to be made at any target DNA sequence by changing the sequences in the guide RNA that can base pair with the target DNA. Hence this technology is a powerful genome engineering tool allowing sequence specific gene editing [3-5].

DNA target recognition in the CRISPR-Cas system requires a short sequence site known as the proto spacer adjacent motif (PAM) that is adjacent to the target sequence on the DNA of interest. The most commonly used Cas9 endonucleases is adapted from Streptococcus pyogenes and it has been optimized for its use in gene editing. The Cas9 has been engineered to derive variants that can increase specificity and cleaving and can be used for wide variety of applications [6].

Cas9 has a HNH nuclease domain that is required to cleave the target strand and RuvC-like nuclease domain that cleaves the non-target strand. By mutating any one of the two domains results in a nickase Cas9 (nCas9) that can now only cleave one strand. This feature is being explored to increase the specificity and cleaving ability. By mutating both the domains of Cas9, an endonuclease which retains the RNA guided DNA binding ability but has lost the cleaving ability is generated. This form of Cas9 called the dCas9 can be used in epigenetic regulation studies where it can be used to various effects or to mediate site-specific regulation without any target DNA cleaving [7-9].

CRISPR-Cas systems have found applications in high-throughput screening, disease target screening and validation, genome imaging etc. [10-20]. The future holds a great promise in deciphering processes that were previously elusive in pathways of cancer and in disease pathogenesis.
Genome editing by CRISPR-Cas9 RNA guided Cas9 is an endonuclease that is directed to the target DNA and creates a site-specific double stranded break that is then repaired by either Non-Homologous End Joining (NHEJ) that can create Indels or Homology Directed Repair (HDR). In the latter type, a donor DNA oligo can be used to precisely edit the DNA.

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