عنوان مقاله:

Trends in CRISPR Animal Genome Editing

محل انتشار:

سومین جشنواره ملی و کنگره بین المللی علوم و فناوری های سلول های بنیادی و پزشکی بازساختی (سال: 1397)

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خلاصه مقاله:

Background and Aim: The CRISPR-Cas9 system is a revolutionarygenome editing technology which has impacted almost every field ofbiological research. During the past 5 years, the research community hasrealized that generating knock-in and conditional knockout models usingthe CRISPR-Cas9 system, via direct mouse zygote injections, is extremelyinefficient. Even though CRISPR-Cas9 tool is very efficient in creatinggene disruptions via nonhomologous end joining repair mechanism, oneof the major problems this tool is the very poor ability to insert foreignsequences at the Cas9 cleavage sites via homology-directed repair(HDR) mechanism. Based on the experience of embryonic stem (ES) celltargeting methods, using double-stranded DNA mediated homologousrecombination (HR), the research community tried employing dsDNAdonor approaches using the CRISPR system. Even tough HR using dsDNAwas successful in a very few cases at an efficiency of about 1 to 10%, ithas been largely unsuccessful for many loci.Methods: In order to solve this major challenge of the CRISPR tool (i.e.,very poor efficiency of insertion of foreign DNA cassettes) we tested twodifferent formats of guide RNA (such as in vitro transcribed single guideRNA or crRNA+tracrRNA), two forms of Cas9 (Cas9 mRNA or Cas9protein) along with long single-stranded DNA donors to test what formatsproduce better knock-in efficiencies.Results: Our results indicate that crRNA +tracrRNA + Cas9 protein alongwith long ssDNA donors yield highest and reliable efficiencies of insertingforeign DNA cassettes at the CRISPR cut sites. We call this method asEasi-(Efficient additions with ssDNA inserts) CRISPR. We show for over adozen loci that Easi-CRISPR generates correctly targeted insertion allelesat a very high efficiency.Conclusion: Easi-CRISPR solves one of the major problems of animalgenome engineering, namely the inefficiency of targeted DNA cassetteinsertion.In my presentation I will discuss how Easi-CRISPR has simplified theprocess of creating designer animal models. I will also present a fewexamples of designing animal models using Easi-CRISPR technology.

کلمات کلیدی:

CRISPR-Cas9; Easi-CRISPR; Knock-in mice; Transgenic mice; Genetic engineering