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SEMINARIO: "Applying an optimized CRISPR/Cas9 system to uncover genes involved in human diseases"

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Abstract

The human genome sequence was the entry point to understand the genetic bases of human diseases. Large-scale genome-association studies have further identified candidate genes associated with human diseases. Sharing high genetic similarity to humans, zebrafish is an excellent model organism to study the function of these genes and more generally vertebrate development. To this aim, genome editing systems to generate genetic models are essential.

Recently, the CRISPR/Cas9 has recently emerged as a genome editing system. This targeting system is based on two components: a single guide RNA (sgRNA) that directs the Cas9 endonuclease to the target site to be mutated. However, the rules determining CRISPR/Cas9 targeting efficiency *in vivo* remain largely unknown.

Here, we have carried out a large-scale analysis of the CRISPR/Cas9 activity *in vivo* using zebrafish embryos. This analysis revealed underlying sgRNA nucleotide preferences affecting CRISPR/Cas9 activity specifically *in vivo*. These novel findings have been integrated into a predictive model (CRISPRscan http://www.yale.edu/giraldezlab/Crisprscan.html). In addition, we have optimized a novel version of the CRISPR/Cas9 system that concentrates the mutations in the germ cells avoiding possible toxicity or lethality coming from mutations in the soma.

This novel system allows for carrying out functional genetic screens in vertebrates in a rapid and efficient manner. Indeed, using our optimized CRISPR/Cas9 system in zebrafish, we identified a novel gene involved in vertebrate brain development that is mutated in a consanguineous family with primary autosomal recessive microcephaly (MCPH). MCPH is a rare neurodevelopmental disorder in children that results in decreases in cognitive abilities and a significant reduction in brain size. The uncovered gene has been

involved in splicing in yeast and human cell lines but not well characterized in any *in vivo* vertebrate system. Currently, we are studying the molecular role of this gene and its relationship with brain development.

In summary, our data provide novel insights into the determinants that mediate CRISPR/Cas9 efficiency and its application to uncover genes involved in human development.

References

Langheinrich U. (2013). Zebrafish: a new model on the pharmaceutical catwalk. Bioessays 25: 904-12.

Hsu, P.D., Lander, E.S., and Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. Cell 157, 1262-1278.