A crispr-cas9 monograph: the potency of targeted genome modulation in vivo

Business

Preface In recent years, with the rise of numerous genetic disorders and autoimmune diseases, researchers have pioneered the CRISPR-Cas9 system as an economical, convenient, and scalable gene therapy treatment. Originally modeled, or identified, in Haloferax mediterranei by Francisco Mojica PhD., at University of Alicante in 1992, the CRISPR-Cas9 is effectively able to modify DNA in vivo (Ledford2, 2017). For example, studies abroad have selectively altered mutations in the MYBPC3 gene to help patients with embryonic hypertrophic cardiomyopathy. It is important to note that just as this system will largely impact scientific community after its formal approval, so too will it affect much of the legislation, business, commerce, and lifestyle of the future of the biotechnology industry.

This monograph assesses the microscopic and macroscopic features of the CRISPR-Cas9 system meticulously to determine the potential of this experimental field. Keywords: CRISPR, Cas9, Gene Therapy, Cancer, Epigenetics, Biotechnology, DNA, Medicine, Monograph, Science, Disease, Bacteria, Autoimmunology, Bioethicality Microscopic & Macroscopic Features At the core, the CRISPR loci is an adaptive immune system for bacteria against phages. Bacteriophages, unable to replicate independently, insert virulent DNA called prophage into a bacterium host’s genome for progeny stimulation by hijacking capsid protein expression. Then depending on population conditions, phages like the Enterobacteria phage ?, may or may not lyse the host cells. After lysis, however, cells have irremediable plasma membrane damage and experience necrosis (Louwen, Staals, Endtz, van Baarlen, & van der Oost, 2014). This phenomenon is observed in nearly 40%

of sequenced bacterial genomes and 90% of sequenced archaeal genomes (Grissa, Vergnaud, & Pourcel, 2007).

The CRISPR or Clustered Regularly Interspaced Short Palindromic Repeat loci allows for guided-RNA endonuclease modulation of genes so that bacteriophage cannot take those initial steps in seizing the cell. Scientists believe that it is an evolutionary defense mechanism of prehistoric archae for species survival (Hille & Charpentier, 2016). Ancient archaea and bacteria that managed to defend themselves against phages would save a portion of the viral complementary double-stranded deoxyribonucleic acid (cdsDNA) in a CRISPR archive. After a succeeding phage infection, they would transcribe a Cas9 endonuclease capable of cleaving viral cdsDNA, by and large, leaving lambda-phages rendered void (Orlova, 2017). The exact mechanism of the CRISPR-Cas loci in prehistoric times has not been fully understood, in spite of that, biologists are making it their primary objective to elucidate more on this currently. A largely accepted hypothesis is that today’s transcribed Cas9 endonuclease is able detect complements in specific genome sites through an crRNA search string (Hsu, Lander, & Zhang, 2014).

Between these specific sites would lie palindromic repeats to spread apart the crRNA, in turn these would aid in the protection of the cross-linkage between the two strands. The specificity of CRISPR-Cas9-mediated cleavage necessitates target sequences match crRNA and a protospacer adjacent motif located downstream of any target sequences (Ledford1, 2017). Humans have been genetic engineering for thousands of years. Through selective breeding, humans have been able to take advantage of

evolutionary adaptations in plants and animals; hence, the concept of changing biotic cells is not novel. Moreover, Zinc Finger (ZFN) and Transcription Activator-Like Effector Nucleases (TALEN) for decades have equally attempted to mimic naturally-occurring transcription artificially. However, the CRISPR loci is different in that it is programmable and universally compatible to any cell type.

Which is something ancient selective breeding methods, ZFNs, and TALENs simply can not control. ZFNs and TALENs are built on protein-guided DNA cleavage, which needs complex and time-consuming protein engineering with a high plausibility of mutagenesis. In contrast, CRISPR-Cas9 only needs a short programmable crRNA for targeting (without any mutagenesis), which is relatively economical and facile to reproduce. Via Cas9 and several crRNA target sites, CRISPR-Cas9 is able to simultaneously induce genomic modifications at multiple discrete positions. This technology has accelerated this generation’s transgenic animals with gene mutations, and has disrupted gene families to investigate epistatic relationships (Zhang, Wen, & Guo, 2014).

Lastly, it would be additionally worth noting that CRISPRhas quite a highly efficient transformation competence rate, unlike much of its competitors. Simply said, the potency is undeniable. It is a far more cost-effective and effortless approach to editing whole genomes. In some studies it was found that CRISPR pushed maximum efficiency curves to their limits in terms of cost-reduction to the standard recombinant DNA technologies and could be completed in just a couple of days with a few dollars (D’Agostino, Locascio,

Ristoratore, Sordino, Spagnuolo, Borra, & D’Aniello, 2017). CRISPR-Cas9 also offers the ability to be selectively programmed, as previously mentioned, novel to the eyes of many geneticists. Also, much like the Yamanaka transcription factors OCT4, SOX2, KLF4, C-MYC, Cas9 can induce nuclear reprogramming by differentiating bacteria, fungi, and archaea into various cell potencies (multi-, toti-, uni-, pluri-, etc.

) as well. This is just an additional utensil in CRISPR’s plethora of abilities. Another distinct attribute of this system is that it is also self-sustaining in vivo, unlike Conan and Boyer’s renown coaxed recombinant plasmid in vitro DNA technology. Furthermore, the loci has yet to find limits in its applications either. CRISPR’s multitude of uses may be exploited for enhancing viral resistance in domesticated microbes, furthering epidemiological studies, building specific immunity against undesirable genetic elements, and conducting host-virus ecological assays. In fact, in a recent epigenomics study conducted by a team, at the Center for Induced Pluripotent Stem Cell (iPSC) Research at Kyoto University, found precise correction of the dystrophin gene in Duchenne Muscular Dystrophy (DMD) via CRISPR-Cas9 modulation.

After targeting a specific annealing site in the human genome through k-mer sequencing, the team demonstrated genetic correction of the dystrophin gene in patient-derived iPSCs by using three different methods: disruption of the splicing acceptor to skip exon 45, introduction of small indels to modulate the protein reading frame, and knockin of the missing exon 44 to restore the full protein coding region. The study also demonstrated the

controlled nature of this biomechanism with no severe off-target, unintended mutagenesis. (Li, Fujimoto, Sasakawa, Shirai, Ohkame, Sakuma, Tanaka, Amano, Watanabe, Sakurai, Yamamoto, Yamanaka, & Hotta, 2015). No doubt, this chimera-like apparatus and efficacy, alike, in the loci give way for the exponential advancement of such genetic, autoimmune, and chronic disorders/diseases. Even with such high esteem and momentum, CRISPR-Cas9 has its fair share of skeptics. On a macroscopic scale, this loci involves high-risk, high-yield manipulation.

Since it may elicit deleterious off target epigenetic mutations, as DNA sequences are often homologous in nature, some geneticists have created a stigma around its application. Just as it has the potency to eradicate any disease, so too can CRISPR develop novel genetic disorders or autoimmune diseases. Under subpar reproduction, if performed incorrectly, it can have life-threatening repercussions not only in one organism, but rather in an entire cladistic lineage of a species (Sander & Joung, 2014). Unchecked reproduction of the CRISPR-Cas9 technology overseas even in today’s world poses numerous ethical implications regarding the formation of unnatural germline, which have swarmed researchers with adversity. As such, the reproduction and subsequent industrialization should be painstakingly detailed. This is so when commercial applications of CRISPR come along, the regulation and legislation surrounding its manufacturing ought to be unequivocal and well-administered.

Another point to make is, in a recent article-review published by the Journal of Clinical Research and Bioethics, the author discusses the possibility of

non-therapeutic interventions using genome editing; for example, the intervention and enhancement of somatic cells to tailor genetics to our fancies and the accidental release and growth of experimentally modified organisms (Rodriguez, 2016). Thus, another pertinent matter in question of are “ designer babies”. Designer babies being a term first coined in Aldous Huxley’s Brave New World (1932) in reference to, metagenetic modification of a human embryo based on a parent’s guidelines by a Preimplantation Genetic Diagnosis (PGD); it has certainly become a sphere ofheated controversy. Of all the past eugenics experiments, this one has had the most significant influence on our world’s future. Cynics upon cynics, critique the idea of unnatural germline production.

Many nations have not yet legislated on genetic modification in human reproduction, but of those that have, all have banned it. Notions of using CRISPR-Cas9 for human reproduction have been largely rejected in principle by the medical research community (Charro & Greely, 2015). So although there may be highly efficacious technologies at our fingertips, there are still some drawbacks to be accounted for. Nevertheless, there are forecasts of hope for designer babies as disorders such as Autism with physiological teratogens will in all likelihood be cured only by the (de)activation of certain genes via CRISPR-mediation. Notwithstanding the arguments made above, targeted genome modulation has its effects both at the macroscopic and microscopic level. With any experimental theory like the CRISPR-Cas9 complex there will always be drawbacks and impediments just as there is promise of progress.

People calling for the end of CRISPR may be named myopes or even defenders of God’s will. Regardless, CRISPR may just prove to be the most revolutionary discovery in medicine since the formulation of Alexander Fleming’s Penicillin Antibiotic. In a nutshell, as our world inches closer and closer to brink of extinction by disease, humans need to find ulterior genomic modulation tools in vivo. Through more clinical research together with federal funding, the world may begin to see perquisites of CRISPR as in the example of the MYBPC3 gene to help patients with embryonic hypertrophic cardiomyopathy (Ledford2, 2017). CRISPR is at its inception, but through trial-and-tribulation, this loci can become a reality we all will come to live alongside.