Lab On A Chip Template

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Abstract

Cancer is caused by a series of alterations in genome and epigenome mostly resulting in activation of oncogenes or inactivation of cancer suppressor genes. Genetic engineering has become pivotal in the treatment of cancer and other genetic diseases, especially the formerly-niche use of clustered regularly interspaced short palindromic repeats (CRISPR) associated with Cas9. In defining its superior use, we have followed the recent advances that have been made in producing CRISPR/Cas9 as a therapy of choice. we also provide important genetic mutations where CRISPRs can be repurposed to create adaptive immunity to fight carcinomas and edit genetic mutations causing it. recently the use of CRISPER-Cas 9 has incresed tremendously compared with other of the endonucleases for example ZFNs and TALENs. challenges to CRISPR technology are also discussed with emphasis on ability of pathogens to evolve against CRISPRs. which can efficiently deliver it to target cells; furthermore, analogous technologies are also discussed along CRISPRs, including zinc-finger nuclease (ZFN) and transcription activator-like effector nucleases (TALENs). Moreover, progress in clinical applications of CRISPR therapeutics is reviewed; in effect, patients can have lower morbidity and/or mortality from the therapeutic method with least possible side-effects. A numer of in depth reviews have been covered adaptive immunity in bacteria, which involves the mechanism of adaptive resistance based on crisper-cas system.

Introduction

Definitions of rare disease are internationally variable, often defined in terms of prevalence and sometimes further qualified with reference to conditions that are 'life threatening' or 'chronically debilitating'. WHO figures suggest that 8% of the world's population are affected by rare genetic disease and there are between 6000 and 7000 rare diseases worldwide. With 80% of rare diseases thought to be genetic in origin and many related to single gene disorders, a focus on rare disease has long been part of genetic medicine. In recent years, available therapies for cancers have been evolving to the betterment of prognosis in patients. Chemotherapy, radiotherapy and surgery are used in combination to reduce the cancerous cells to remission, that increases lifespan to a maximum of five years. However, harmful side effects and toxicity increases the mortality whilst it significantly reduces the quality of life . The understanding of cancer biology is of key importance to develop novel anti-cancer therapies. The present day advances in sequencing technology have helped to explore the cancer genome more efficiently with much lower cost. Cancers are characterized by DNA and RNA alterations including mutations, gene duplications and changes in messenger RNAs. The integrative approach to utilize genomic and transcriptomic advances can unveil the complete picture of individual genome. The understanding of cancer has been revolutionized by the present day next generation sequencing technologies . The NGS provides the identification of specific mutations relevant to cancers and other genetic diseases at genomic level that can be edited by genome editing technologies the ZFNs, TALENs and CRISPRs or the combination of them. The NGS technologies include whole genome sequencing, whole exome sequencing, RNA sequencing, reduced representation bisulfite sequencing, and chromatin immunoprecipitation sequencing each of which is employed for specific objectives. In cancers often the whole exome

sequencing is performed to get specific mutations at the cellular levels.

GENOME EDITING TOOLS:-

The modifiable ability of genome editing nucleases to make specific double stranded DNA breaks (DSBs) which are primarily repaired by naturally present nonhomologous end joining (NHEJ) DNA repair pathway that is prone to frame-shift mutations resulting in gene disruption . This condition can be precisely corrected by providing a template along with nucleases that will follow a homologous repair (HR) pathway to mend DSB. It has been previously reported that a nuclease induced DSB near a disease mutation can significantly enhance HR pathway . Hence it is very much possible to correct the mutated gene by providing a template of wild type gene thus greatly enhances its applications in biomedical research.

ALTERING Epigenome with crispers/ cas9

Epigenetic modifications consisting of DNA methytlation and histone modifications provides an essential environment for stimulating gene expression that defines their cell proliferation and differentiation activity. Histone proteins responsible for packaging the whole DNA in eukaryotic cells undergo several epigenetic modification including ubiquitination, phosphorylation, SUMOylation, and acetylation. All those are of reversible nature and are under the control of epigenetic modification enzymes These epigenetic covalent modifications of histones are of high importance in repression or activation of gene expression . Apart from histone modifications, the structure of chromatin is also defined by DNA methylation. The first ever epigenetic modification identified is carried out by DNA methyltransferase enzymes (DNMTs), that provides environment to prevent binding of transcription factors and/ or bring repressive protein complexes to DNA . DNA methylation is more stable in comparison with post translational modifications of histone but can still be demethylated by active and passive mechanisms and is responsible for the normal development and cellular differentiation . These days several non coding RNA (ncRNA) species for example micro RNA (miRNA), short interfering RNA (siRNA) are found to inhibit or activate genes that are involved in the epigenetic regulation of critical biological processes of growth and development. miRNAs are implicated in several disease conditions and can be used to target specific gene expression for its up and/or down regulation as required . Recently, CRISPR/Cas9 has been successfully employed to edit genetic switches and many of the miRNAs that are involved in cancer progression and development can be specifically targeted with CRISPR/ Cas9 genome editing system.

CONCLUSIONS

There is much buzz around genome editing technologies specially CRISPRs to be used against several life threatening diseases at the molecular level. The phrase “Nip the evil in the bud” may rightly be used for CRISPRs therapeutics but it is critical to develop all the requisite clinical tests for its efficacy, safety and specificity before its use in clinics. The short journey of CRISPRs till now is highly fascinating and provides a significant hope of medical cure against deadly diseases.

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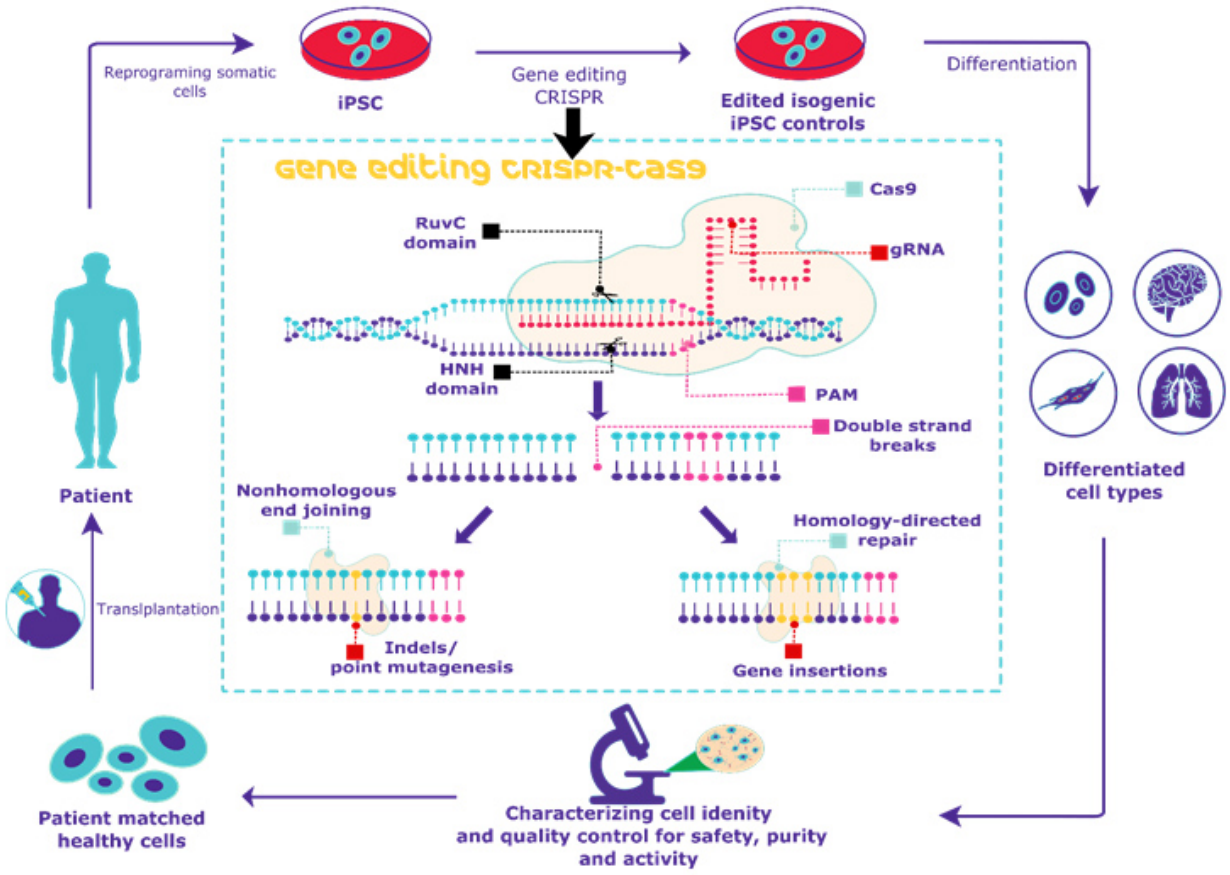
Reprograming somatic cells
iPSC
Gene editing CRISPR
Edited isogenic iPSC controls
Differentiation
Gene editing CRISPR-Cas9
Cas9
RuvC domain
gRNA
HNH domain
PAM
Double strand breaks
Nonhomologous end joining
Homology-directed repair
Indels/ point mutagenesis
Gene insertions
Patient
Transplantation
Differentiated cell types
Patient matched healthy cells
Characterizing cell idenity and quality control for safety, purity and activity

References

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