**[The molecular mechanism of CRISPR/Cas9 system and its application in gene therapy of human diseases]**

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Abstract:CRISPR/Cas system is an adaptive immune system, and it can resist exogenous virus or plasmid in bacteria. From 2013, the CRISPR/Cas9 genome editing technology modified from typeⅡCRISPR/Cas adaptive immune system has been applied to many research fields of life science,and as a result,the application has invoked remarkable changes.[1] In this article, it summarizes the origin and development of CRISPR/Cas9 genome editing technology,and of course, its applications in the research. Meanwhile, the associated side/off-target effects are also mentioned, which may be meaningful to scientists in certain fields.

**Introduction**

CRISPR is a special DNA sequence family,which is Widely distributed in the genome of bacteria and archaea.The CRISPR system has 3 different types(type 1,type 2,type 3).Here we will only talk about TYPE 2. There is only one kind of nuclease in this system,that is,CAS9,which gets involved in the resistance of invasion of exogenous phage and plasmid together with crRNA((CRISPR RNA).

The mechanism is shown below. CRISPR completes the identification of exogenous DNA through ‘spacers’(26-72bp),which separate the highly-conserved sites(21-48bp). CrRNA through base pairing with tracrRNA (Trans-activating crRNA) node close and form a double-chain RNA. This tracrRNA/crRNA binary compound guides Cas9 protein to make cleavage of DNA at a specific site, which the HNH nuclease domain of Cas9 cuts complementary chains, and the RuvC-like domain cuts non-complementary chains.Recently,the binary compound is proved that it can be replaced by sgRNA(Single-guide RNA),a target-directed RNA.

Sometimes,when the sgRNA guides Cas9 protein to make cleavage of the specific DNA,it leads the tail of the double chains to break apart.Then the cell can fix it using HDR or NHEJ method. When using NHEJ, the deletion and insertion of the bases in the junction can lead to the mutation in the open reading frame,thus causing the target genes to be unactivated, and the genes’ knockout can be realized. When cells have exogenous DNA gene homology arm, it can realize the exogenous gene typing by HDR pathway

Considering the properties above,the system has many applications in cancer research, genetic diseases,genetic therapy,and so on.

**Recent Progress**

1.Application of CRISPR/Cas9 system in genome editing

The review cite many examples in genome editing using this system,and here I will introduce a few of them.

Yuan Jing and other researchers from Xiamen University successfully complete the deletion and knock-in of genes and the replacement of nucleotide using Cas9 genome editing technology.The researchers use electroporation to transfer sgRNA,Cas9 and donor with homologous arms into the nucleus of Malaria Parasites.They successfully delete genes which are 1.7 kb,4.0 kb and 5.0 kb large.[1]In addition,they find longer the arms,larger the probability of gene deletion.Their work is of great importance,because malaria is a heavy burden in the world,especially in some African countries.

In Yuan’s article, the reaearchers report the development of a method based on the CRISPR/Cas9 system to efficiently edit the genome of Plasmodium yoelii, including KO, KI, or AR multiple P. yoelii genes with high efficiency and accuracy.[2]Firstly,they construct the Cas9 nuclease and targeting single guide RNA (sgRNA),in order to induce a double-strand break (DSB) at the targeted site.Next,when injected into the cell,the system comes into function.After homologous repair,the deletion,knock-in and replacement are all accessible.[2]

2.Application of CRISPR/Cas9 system in gene therapy for hereditary diseases

In August 5th,2014,researchers from University of California reported that they are now able to induce skin cells form patients suffering from β-Thalassemia into iPSC (Inducible pluripotent stem cell),then they use CRISPR/Cas9 technology to repair the abnormal HBB gene,these repaired iPSCs can be turned into normal erythrocytes,which can express normal HBB proteins.[1]

To correct the mutation of -28A/G and TCTT deletion at HBB in the b-thalassemia iPSCs, they constructed a targeting donor plasmid by amplifying two 500-bp segments of the genomic sequences upstream of and downstream from the TTAA sequence at intron 1 of HBB.[3]Then,almost the same principle mentioned above.After a series of analysis,they confirmed that HBB genes can be efficiently restored in this way.

3.Application of CRISPR/Cas9 system in gene therapy for viral infectious diseases

In November 4th,2013,a review from virus talks about using CRISPR/Cas9 technology to treat HIV. Bone marrow stem cells, which are genetically modified, can differentiate into CD4+T cells that produce resistance to HIV-1 infection.[1]

In fact, ZFN and TALEN can contribute to the treatment too.But unlike ZFN and TALEN, CRSPR/Cas9-mediated genome editing system adopts a Watson-Crick complementarity rule via a short RNA molecule that is homologous to the target site.[4]

However,there are too many copies of the ex-genome in the HIV-infected,so the Cas9 nuclease may not delete all of them,and that can be worrying.

4. Application of CRISPR/Cas9 system for cancer treatment

In order to know the mechanism of cancer,scientists sometimes need to create some models.In 2014,it is firstly reported the application of CRISPR/Cas9 in construction of cancer models. [1]Under the guidance of specific Cas9, DNA is cleaved at the special site of the chromosome, which causes the chromosome to be inverted and ectopic.

Molecular analysis of the breakpoint junctions confirmed that accurate DNA-end joining was taking place (Fig. 4d and Supplementary Fig. 6), and RT–PCR analysis of mRNA confirmed expression of the RUNX1/ETO fusion gene derived from the chromosomal translocation.[5]

In addition to the applications mentioned above,there are still many useful ones,which I will not talk about here.

**Discussion**

The examples above convince me that the CRISPR/Cas9 system may be a key to solving problems like genome editing.However,it also has some deficiencies,like off-target effect.It means the sgRNA doesn’t guide Cas9 protein to the right site,which may lead to the mutation of other sequences,activation of cancer genes,and so on.

What’s more,it is really difficult to sufficiently transfer Cas9 genome-editing plasmids into the desired cells,tissues and organs.In vitro,we can use to electroporation to transfer Cas9 genome-editing plasmids into the nucleus,but it can be harmful to the cell,lowering its vigour,so it may not achieve the goal we want. So maybe we can use viruses to transfer,like AAV.Also,nanoparticle and lipidosome can be promising choices.

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