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CRISPER/Cas9 as a Promising Tool for Development Malaria Vaccine

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Review Article

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Abstract

With regard to the transportation and economical exchanges, vector borne diseases should be considered as potential risks for different countries that have the suitable situation for their emergence and distribution. Malaria is one of them that numerous countries have been involved in challenge with this disease from previous years. Vaccines have been the first choice for preventing and controlling these types of diseases, but despite the performed efforts for developing the malaria vaccine, there is no a preventive vaccine for this disease. Therefore, this subject is on the focus of researchers to design an efficient vaccine for combatting against malaria. According to the plasmodium parasite life cycle, different types of malaria vaccines have been introduced that one of them are attenuated parasites. The first strategies for creating attenuated parasites were radiation. By revolution in genomics approaches, a breakthrough was occurred in this type of vaccine development. Functional genomics helps to determine the gene functions and by homologous recombination, it is possible to create specific mutation or gene knock out. Between different methods, CRISPR/Cas9 is an efficient method for gene targeting. In this review, a brief description of CRISPR/Cas9 is presented.

Keywords: CRISPR/Cas9; Pre-Erythrocytic; Sporozoite

Introduction

Despite the significant efforts for controlling and eradicating, malaria parasite infections constitute extreme challenges and still is the third cause of mortality worldwide [1]. Based on the recent WHO report in 2018, an estimated 228 million cases of malaria occurred worldwide. Most malaria cases in 2018 were in the African countries (213 million or 93%). Globally, there were an almost 405,000 deaths due to the malaria infection. The most vulnerable group, who are affected by malaria, is children aged less than 5 years. In 2018, around 11 million pregnant women were exposed to malaria infections and it was resulted to the near 872,000 children with low birth weight. In addition, it was estimated that approximately 24 million children were infected with P. falciparum in 2018 in sub-Saharan Africa, and 1.8 million of them had severe anemia [2]. Consequently, development of the effective vaccines that reduce the malaria incidence has been emphasized by world health organization (WHO) as one of the important preventive solutions [3]. Several types of malaria vaccines have been introduced which are including pre-erythrocytic vaccines, antibody-based subunit vaccines, vector based vaccines [4], whole sporozoite vaccines, genetically attenuated parasites (GAP) and sporozoite subunit vaccine, erythrocytic vaccines, sexual stage vaccines, transmission-blocking vaccines (TBVs) as well as the synthetic peptides and conjugate vaccines [5,6]. In general, vaccines are divided to two groups: attenuated or killed pathogen or microbes and protein subunits or conjugate vaccines [7]. Although development of malaria

vaccines has been faced with many challenges, there have been remarkable progresses as well [8,9]. Whole sporozoite vaccines [10] include the Radiation-attenuated P. falciparum sporozoites (RAS) [11], Chloroquine Prophylaxis and Sporozoites (CPS) [12] and Genetically Attenuated Parasites (GAP) [13]. The last member of this family (GAP) has gathered the attentions of many researchers for developing a robust and effective vaccine [13,14]. The most important tools in this subject are homologous recombination approaches such as zinc-finger, TALEN and CRISPR/Cas9 [15,16]. According to the performed studies, CRISPR/Cas9 is more efficient and simpler rather than other methods and it has been used for malaria vaccine development in recent years in several studies [17]. In this paper, we review these methods and indicate some performed studies.

Malaria Vaccine

RAS

Inducing immunization via the gamma radiation-attenuated sporozoites (RAS) which is a Pre-erythrocytic (PE) vaccine leads to a complete protection. Through this way, radiation damages the DNA in sporozoites and makes them non-infective. Although it produces a high protection in human, this method is not cost-effective and not practical on a large scale [11,18,19].

CPS

Another strategy for immunization with whole sporozoites is chloroquine prophylaxis (CPS) that prevents the blood stage development [20] and it has no effect on sporozoites and liver stages [12,21].

GAP

Genetically attenuated parasites (GAP) are developed by genome edition, stage-specific toxins and gene deletions that are up-regulated in infective sporozoites and produce a long-lasting protection by CD8+ T cells [22]. This method is not relied on specific gene [23-25].

Genetic Manipulation Methods

With regard that P. falciparum and P. berghei could be cultured in vitro, most of the studies have been performed on these parasites. In order to promote the development of malaria vaccine, it is essential to study the Plasmodium falciparum at molecular level and find a highly protective subunit malaria vaccine. Providentially, with the progress of genetic manipulation approaches; it is possible to develop the novel specific genetic manipulated malaria parasites as vaccine [26]. These types of vaccines are developed by targeting the specific genes using the genetic manipulation methods [27,28].

Recombination Strategies

Single or double-crossover recombination strategies, which are occurred by both homologous and non-homologous recombination, have been employed to edit the target genes in P. falciparum [29,30]. Recombination methods were inefficient and needed several months to make the suitable gene modification because plasmids were used for gene targeting and transgene expression like green fluorescent protein expression and various drug selection cycles were required to isolate the parasites with the desired integration [31,32].

Zinc-Finger Nuclease (ZFNs)

Zinc-finger nuclease (ZFNs) is a highly efficient technic that produce a double-strand break (DSBs) in a user-defined locus and trigger homology-directed repair [33]. Although it generates an effective mutation in the target gene, this approach has not been employed in wide range, because of the cost and complexity of the design process.

The CRISPR/Cas9 Genetic Modification

The CRISPR/Cas9 genetic modification tool was extracted from Streptococcus pyogenes and was identified as a prokaryotic defense mechanism [34-37]. It is a precise editing tool which has been applied in several organisms, especially for P. falciparum [38]. This effective system has been introduced as a powerful tool that could be used to silence a target gene or modify its sequence, in order to study the function of genes or modify the organism and change its phenotype. It is the cost-effective and possibly more efficient and faster than the other gene editing methods that could be used for therapeutic and vaccine development purposes by the single guide RNA (sgRNA) and subsequently inducing double-strand breaks (DSBs) that is repaired by homologous recombination [39].

Using the CRISPR/Cas9 Tool in Malaria Vaccine Development

Jinek et al in 2012 showed that Cas9 could be purposed by an exclusive guided RNA, so it precisely cut the gene of interest at the specific DNA site [40]. A transgenic Plasmodium falciparum NF54 strain was created by Vaughan 2012. This strain expressed GFP–luciferase throughout the all parasite life cycle and was completely measurable [41]. In 2014, Ghorbal applied the CRISP/Cas9 genome editing in Plasmodium falciparum for the first time and break chromosomal loci and generate marker-free, single-nucleotide substitutions [42]. In 2014, Cui Zhang, Bo Xiao, Yuanyuan Jiang introduced Cas9 to specific site of DNA to

create double-strand break in the Plasmodium yoelii genome and generated targeted deletion, reporter knock-in, and nucleotide replacement in multiple parasite genes. In this study, 100% efficiency in gene deletion was developed [43].

Another study was performed in 2015 by Valentino M Gantz, et al. [44] on developing a highly effective CRISPR/Cas9 in the Asian malaria vector Anopheles stephensi. They provided an active gene cassette that could spread antimalarial genes into the vector population and limited the germ line with ≥98% efficiency [44].

Junnan Lu in 2016 [45], redesigned a marker-free CRISPR/Cas9 system that needs fewer selectable markers and its efficacy was demonstrated for large gene cassette knock-ins [45].

In 2018, Marin Mogollonet et al. developed specific constructs that had ability to apply in P. falciparum. These improved CRISPR/Cas9 transfection constructs could be used to generate the reporter proteins like fluorescent and bioluminescent or introduce transgenes into the parasite genome without the inclusion of drug-selectable marker genes. In this system, the encoding gene of GFP is stably integrated into the P. falciparum genome under the control of promoters of three different Plasmodium genes (calmodulin, gapdh and hsp70) [46]. In addition, recently in 2019 Marin Mogollonet expressed the reporter lines of cherry and luciferase in gametocytes and sporozoites of P. falciparum. Expression of gene reporter proteins improves the in vitro analysis of transmission blocking inhibitors [47].

Conclusion

With regard to the importance of malaria vaccine development to reach the global eradication, different types of vaccines were considered in this process. Each type has its specific advantages and drawbacks. Among them, attenuated vaccines have been on the focus of the researchers from previous years. Recently, new type of the attenuated vaccines has been developed as genetically attenuated parasites. The advantages of this type of vaccines is that those are immunogenic intrinsically and no need for specific adjutant and formulation and those proceed the asexual parasite life cycle in human body till the specific stage according to the targeted manipulation. By revolutions in functional genomics and related approaches, the role of different genes in pathogenesis have been determined and these data pave the road for creation of specific mutant parasites. The arms of this strategy are the genetic engineering methods. CRISPR/Cas9 is a promising and new method which has been introduced as an efficient tool for targeted gene manipulation in different organisms. As discussed previously, this method has been used extremely for genetic manipulation of different species of plasmodium parasites. Therefore, it could be used for mutant parasite creation and using them in malaria attenuated vaccine development.

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