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GUEST COMMENTARY

A Broad Application of CRISPR Cas9 in Infectious, Inflammatory and Neurodegenerative Diseases

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Abstract

Being the most important immune-responsive cell type of the CNS, microglia always glorify the so-called crossroad of Neurology, Immunology and Pharmacology. As microglial activation is a hallmark of different neurodegenerative disorders including Alzheimer's disease (AD), Parkinson's disease (PD), HIV-associated neurocognitive disorders (HAND), Amyotrophic lateral sclerosis (ALS), etc., selective targeting of microglial cell signaling may be a valid option to control these neurodegenerative disorders with lesser side effects. This is particularly important as no effective therapies are available against these diseases and available neuroimmune modulators are known to target multiple cell types in a non-cell-specific manner. How we can achieve such specificity? A newly-developed cutting-edge molecular biology tool is rocking biomedical research in recent years so much so that it has already come under major lawsuits between the University of California Berkeley and the MIT-Harvard Broad Institute regarding its ownership rights, probably halting the Nobel committee to announce the most coveted prize to its owners. It is none other than Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). In nutshell, the Cas9 enzyme has been paired with the bacterial immune system, CRISPR, to ultimately turn CRISPR/Cas9 as an effective genome editor. Therefore, this special issue has been devoted to highlight some of the recent discoveries on CRISPR/Cas9 in neurodegenerative disorders and explain these discoveries in the light of neuroimmune pharmacology.

Keywords CRISPR · Cas9 · Microglia · Neuroinflammation · Neurodegenerative disorders

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Being the most important immune-responsive cell type of the CNS, microglia always glorify the so-called crossroad of Neurology, Immunology and Pharmacology (Gendelman and Mosley 2015; Li et al. 2016). As microglial activation is a hallmark of different neurodegenerative disorders (Saha and

Pahan 2006; Stone et al. 2009; Perry et al. 2010) including Alzheimer's disease (AD), Parkinson's disease (PD), HIV-associated neurocognitive disorders (HAND), Amyotrophic lateral sclerosis (ALS), etc., selective targeting of microglial cell signaling may be a valid option to control these neurodegenerative disorders with lesser side effects. This is particularly important as no effective therapies are available against these diseases and available neuroimmune modulators are known to target multiple cell types in a non-cell-specific manner.

How we can achieve such specificity? A newly-developed cutting-edge molecular biology tool is rocking biomedical research in recent years so much so that it has already come under major lawsuits between the University of California Berkeley and the MIT-Harvard Broad Institute regarding its ownership rights, probably halting the Nobel committee to announce the most coveted prize to its owners. It is none other than Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). In nutshell, the Cas9 enzyme has been paired with the bacterial immune system, CRISPR, to ultimately turn CRISPR/Cas9 as an effective genome editor. Therefore, this special issue has been devoted to highlight some of the recent discoveries on CRISPR/Cas9 in neurodegenerative disorders and explain these discoveries in the light of neuroimmune pharmacology.

Glia maturation factor (GMF), a neuroinflammatory acidic protein, is highly expressed in the brain during insult or injury and has been implicated in PD pathology. Role of GMF in microglial activation is poorly understood. MPP^+ (1-methyl-4-phenylpyridinium) is a parkinsonian toxin and here, Selvakumar et al. (2019) have demonstrated that CRISPR/Cas9-mediated targeting of GMF inhibits MPP^+-induced microglial activation via reduction of oxidative stress, decrease in Ca^{2+} flux and attenuation of NRF2 translocation. They have also demonstrated that GMF knockdown reduces the expression of inducible nitric oxide synthase and cyclooxygenase 2 in MPP^+-stimulated microglial cells. These results highlight suppression of microglial activation by CRISPR/Cas9-mediated GMF-gene editing and suggest that GMF may be a possible target for therapeutic intervention in PD.

It is known that cigarette smoke makes flu and other viral infections worse. This is mainly due to suppression of antiviral response by nicotine, the primary addictive component of cigarette. IRF7 is the master regulator of the type I IFN-dependent antiviral innate immune response. Since the underlying mechanism behind nicotine-mediated antiviral response was not known, here, Han et al. (2019) have investigated whether IRF7 couples nicotine to antiviral response. Therefore, by using the CRISPR/Cas9 system, they have established two IRF7-mutant cell lines of HEK293FT with 7- and 11- nucleotide deletions to demonstrate that double-stranded RNA (poly I:C)-induced elevation of the genes related to the antiviral response is decreased in IRF7-mutant cells as compared to WT cells. These results suggest that IRF7 plays an important role in nicotine suppression of innate antiviral immune responses and that IRF7 antagonists may have implications in correcting the antiviral immune response in cigarette smokers.

Since CRISPR/Cas9 system shows promise for different neurodegenerative disorders, proper delivery of this gene editing tool to the correct target in the CNS is an important area of research as off-target mutations and immunogenicity are always valid concerns of CRISPR gene editing. Therefore, in an encyclopedic review, Campbell et al. (2019) discuss pros and cons of an established approach of CRISPR/Cas9 delivery using a pre-formed ribonucleoprotein (RNP) Cas9 + gRNA complex. They have described possible alternative delivery methods of CRISPR/Cas9 (e.g. delivery of Cas9/RNP complex by cell-penetrating peptides, nanoparticles, microvesicles, etc.) that may be helpful in translating this technology into therapies for neurodegenerative disorders. They have also provided interesting data to suggest that microvesicles have potential as method to deliver Cas9 RNP's targeting the HIV provirus in microglia populations in vivo in the brain.

Although the therapeutic potential of CRISPR/Cas9 system lies in its unique ability of genomic DNA editing, the CRISPR/Cas9 system has been applied to target multiple human pathogens in vivo and in vitro system. Virus-induced neurological abnormalities are associated with significant morbidity and mortality. Here, Bellizzi et al. (2019) describes how viral genome editing by CRISPR/Cas9 may be an effective and a highly specific tool for targeting CNS viral infection. Although anti-retroviral therapy (ART) can successfully control HIV infection, it is unable to affect latently infected cells that ultimately serve as the source of reemerging viruses. The inability of either ART therapy or the immune system to recognize and destroy these cells represents a major challenge for HIV eradication. This review also touches this important issue and describes how catalytically-deficient Cas9-synergistic activation mediator technology has been used to activate HIV from latent viral reservoirs.

PD is the most common neurodegenerative movement disorder in humans and until now, no effective therapy is available to stop the progression of this disease. Here, Luo et al. (2019) have nicely described how the CRISPR/Cas9 technology is transforming PD research through the identification of novel apoptotic pathways associated with neurodegenerative processes in PD, dissection of PD-associated neuroinflammatory as well as protective or compensatory pathways, repair of PD-associated genetic mutations, etc. Moreover, development of appropriate animal model is key to the development of different therapeutic approaches against any human disease. This is particularly important for PD as there is no good animal model to mimic the PD-associated progressive nigrostriatal pathology. Here, they have also

delineated how CRISPR-Cas9's precise genome editing is facilitating the generation of innovative animal models of PD (e.g. Parkin, DJ-1 and PINK1 triple knockout pig model).

Age-dependent neurodegenerative disorders are becoming increasingly common, partly because of increase in elderly population in recent years. Therefore, there is an urgent need to understand underlying mechanisms and develop new and more effective therapeutic strategies to combat these devastating diseases. Here, in a thoroughly-described review, Raikwar et al. (2019) have described how targeted genome editing holds a tremendous potential to decipher the complex molecular mechanisms underlying neuroinflammation and neurodegeneration in a number of neurodegenerative disorders. They have also described possible utilization of CRISPR/cas12a, CRISPR/Cas13 and TUNR flexible gene editing technologies in neurovirology and neurodegenerative disorders. It is nice to see that Cas13-based SHERLOCK (specific high-sensitivity enzymatic reporter unlocking) platform can detect Zika virus and dengue virus in patient samples at concentrations as low as 1 copy per microliter. Moreover, they have discussed potential roles of SLENDR (single-cell labeling of endogenous proteins by CRISPR/Cas9-mediated HDR) technology in in utero gene editing, the future for heritable lysosomal storage disorders (e.g. Batten disease) and metabolic brain disorders (e.g. X-Adrenoleukodystrophy).

It is not possible to cover all recent developments on CRISPR/Cas9 in the areas of microglial activation and neurodegenerative disorders. Therefore, here, we have made an honest attempt to highlight some of the emerging applications of CRISPR/Cas9 in neuroinflammation and neurodegeneration. Identification and characterization of various neuroimmunological pathways regulating the pathogenesis of neurodegenerative disorders would be important for designing new therapeutic strategies against these devastating diseases. Therefore, we believe that this exciting collection of review and research articles clearly highlighting emerging aspects of CRISPR/Cas9 may pioneer and steer future research efforts aimed to reveal further secrets of CRISPR/Cas9 gene editing and provide promising therapeutic strategies for neurodegenerative disorders in which microglial activation plays a role.

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Compliance with Ethical Standards

Conflict of Interests None.

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