929/D

In silico identification of potential TALEN and CRISPR/Cas9 targets in Hepatitis B viral genome: A predictive approach for the treatment of viral infection.

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Hepatitis B virus (HBV) is a potential viral pathogen of humans and cause chronic infection leading to several health problems such as chronic hepatitis, cirrhosis, and several types of liver cancers. Although the pathogen causes severe health issues, a treatment method to cure the disease is not developed yet and medications are available only to suppress the disease. But there is potential to use the modern gene editing techniques on HBV to eliminate the infection from the human body and several researchers have successfully tested the techniques on the virus. In this research, we selected the *HBx* gene which is responsible for the protein X, to identify potential Transcription Activator-Like Effector Nuclease (TALEN) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) Associated Protein 9 (CRISPR/Cas9) target sites. The gene could thus be mutated, leading to the mutated protein, which might lack the original function. The reason behind the selection of gene *HBx* is that the protein X is involved in the reproduction and persistence of the virus and persistence is the reason for the above-mentioned health problems. Other than that, protein X interacts with many other human proteins, and this interactome was observed, and is available in the tool 'virusmentha'. The TALEN target sites of the gene were identified using the tool 'TALEN targeter' of 'TAL effector nucleotide targeter 2.0'. Several TALEN sites with maximum binding specificity were selected and analyzed for off-target bindings in human genome and for unnecessary bindings of the same viral genome using the tool 'Paired target finder' of 'TAL effector nucleotide targeter 2.0'. Further, the off-target bindings in the human genome was tested using the tool 'PROGNOS' and several TALEN sites for each of eight genotypes of HBV with highest binding affinity and minimal off-target effect were identified. Apart from that some CRISPR/Cas9 target sites, which are common to all the genotypes of the virus, were identified and analyzed for their off-target effect using the tool 'CC-Top'. But for CRISPR/Cas9 many possible off-target bindings in human genome were identified. So TALENs are probably more suitable than CRISPR/Cas9 nucleases for the editing of *HBx* gene. These identified TALEN sites and respective TALENs have the potential to be used as a treatment strategy for HBV but the on-target and off-target effects should be assessed further by *in vitro* and *in vivo* studies.

Keywords: Hepatitis B virus, *HBx* gene, TALEN, CRISPR/Cas9, Bioinformatics

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