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Safer gene editing approaches towards HIV-1 gene therapy

CCR5 gene disruption is a promising method for HIV-1 gene therapy and recent gene editing technologies such as ZFNs and CRISPR/Cas9 provide methods for such disruption. However, one major concern of using nucleases is off-target effects associated with their long-term expression. Thus, successful clinical translation of gene editing strategies necessitates the development of safe and effective methods for their transient expression in relevant cells. We have modified the *ZFN* and *CRISPR/Cas9* gene editing technologies to provide for transient expression of nucleases. We used non-integrating lentivirus (NILV) for transient expression of ZFNs and pseudotyped the virus with HIV-envelope for targeted delivery to CD4 T cells. Both activated and resting primary CD4 T cells transduced with CCR5-ZFNs NILV showed resistance to HIV-1 infection *in vitro*. Furthermore, NILV transduced resting CD4 T cells from HIV-1 seronegative individuals were resistant to HIV-1 challenge when reconstituted into NOD-scid IL2rγc null (NSG) mice. Likewise, endogenous virus replication was suppressed in NSG mice reconstituted with CCR5-ZFN-transduced resting CD4 T cells from treatment naive as well as ART-treated HIV-1 seropositive patients. Taken together, NILV pseudotyped with HIV envelope provides a simple and clinically viable strategy for HIV-1 gene therapy. Since the CRISPR/Cas9 system provides an easier way for gene editing, we also modified this system for transient expression of Cas9 protein. For this purpose, we pre-packaged Cas9 protein (Cas9P LV) in lentiviral particles and showed its effectiveness for gene disruption in cells, including primary T cells expressing specific sgRNAs. We then constructed an "all in one" lentivirus to express sgRNAs in association with prepackaged Cas9 protein (sgRNA/Cas9P LV). We successfully edited CCR5 in TZM-bl cells by this approach. Using a sgRNA targeting HIV LTR, we also were able to disrupt HIV provirus in the J-LAT model of viral latency. Moreover, we also found that pre-packaging Cas9 protein in LV particle reduced off-target editing of chromosome 4:-29134166 locus by CCR5 sgRNA, compared to continued expression from the vector. These results show that sgRNA/Cas9P LV can be used as a safer approach for human gene therapy.

Biography

Manjunath N Swamy is currently Prof. Biomedical Sciences and Co-Director of the Center of Emphasis in Infectious Diseases. He obtained his MD degree from the All India Institute of Medical Sciences, New Delhi, India and received Post-doctoral training at the Tufts-New England Medical Center. He was as an investigator at the Immune Disease Institute, Harvard Medical School before joining the TTUHSC

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