CRISPR-Targeted Genome Editing of Human Induced Pluripotent Stem Cells-Derived Hepatocytes for Treatment of Wilson's Disease

Rui Wei[1,2*]; Jiayin Yang[1,2*]; Wai-In Ho[1,2]; Chi-Wa Cheng[1,2]; Na Li[1]; Wing-Hon Lai[1,2]; Yiu-Lam Tse[1,2]; Kwong-Man Ng[1,2]; and Hung-Fat Tse [1,2]

[1]The Cardiology Division, Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China. [2]Hong Kong-Guangdong Stem Cell and Regenerative Medicine Research Centre, The University of Hong Kong and Guangzhou Institutes of Biomedicine and Health, Hong Kong SAR, China. *These authors contributed equally to this work and are co-first authors.

Abstract

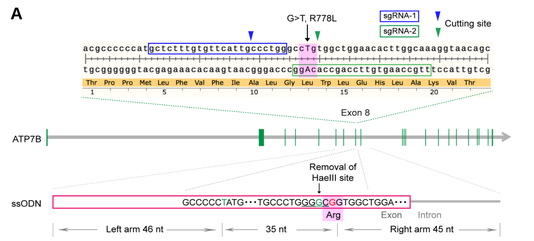
Wilson's disease (WD) is a monogenic liver disorder characterized by dysfunction of copper exportation and abnormal copper accumulation. It is caused by mutation of the Wilson's disease gene – ATP7B, which encodes an essential copper transportation protein. Recent advances in iPSCs and genome editing technologies may enable us to develop autologous cell replacement therapy for WD. In this study, we corrected one of the most common disease-related mutant, ATP7B R778L, in Chinese WD patient-derived iPSCs using CRISPR/Cas9 and ssODNs and differentiated the gene-corrected iPSCs into induced hepatocytes (iHeps). The WD iHeps regained normal ATP7B subcellular localization as well as copper exportation function after gene correction. Moreover, transplantation of gene-corrected WD iHeps attenuated the disease phenotypes of WD mouse models. Our results provided a comprehensive proof-of-principle for employing CRISPR/Cas9 to obtain genetically corrected iHeps for autologous cell-based therapies for WD and other inherited liver diseases.

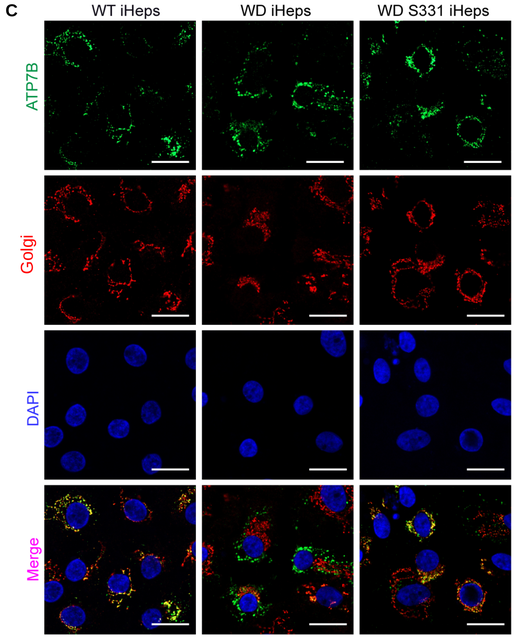
Introduction

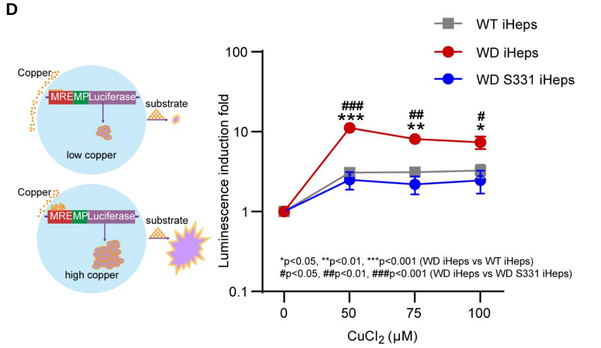
Wilson's disease (WD) is a monogenic liver disorder due to malfunctions of the ATP7B gene (Ala et al., 2007). ATPB R778L is the most prevalent WD mutation in the Chinese population, and predominantly presents as liver disease. Our lab has previously generated WD iPSCs from fibroblasts of a WD patient with homozygous R778L variants (Zhang et al., 2011). Here we aim to study autologous cell replacement therapy for WD by transplanting iHeps derived from our WD patient-specific iPSCs as well as gene-corrected iPSCs. Our results provide proof-of-principle data to show that iHeps derived from gene-corrected WD iPSCs can restore function of the ATP7B gene and are a potential cell source for hepatocyte transplantation therapy.

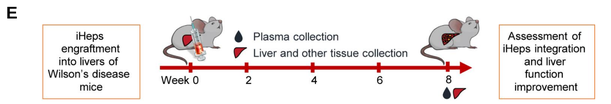
Methodology

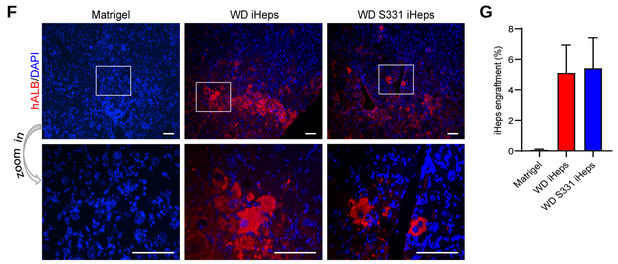
In this study, we corrected the homozygous ATP7B R778L mutation in WD iPSCs using CRISPR/Cas9 and ssODNs. After gene editing, we induced the iPSCs into iHeps (Kajiwara et al., 2012), and further evaluated the recovery of ATP7B functions in-vitro, as well as their therapeutic potential in WD mouse models.

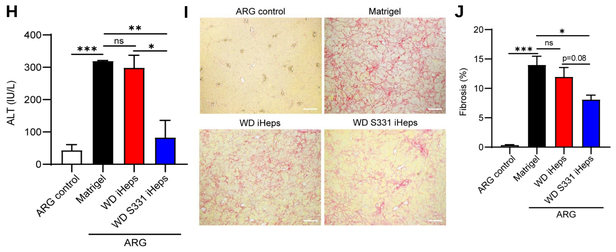


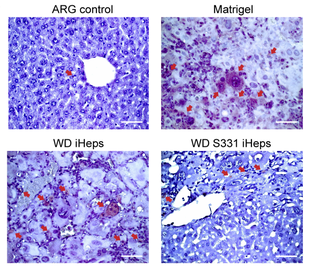


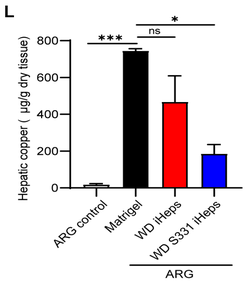












Discussion and Conclusion

Comparing to whole liver transplantation, hepatocyte transplantation is more flexible and much less traumatic. iPSC-derived iHeps bear identical genetic background of the donor and are promising for autologous cell therapy.

By utilizing CRISPR/Cas9 and ssODNs, we corrected ATP7B R778L mutant in a footprint-free manner. This footprint-free gene correction method avoids introducing foreign DNA fragments to the host genome, making the gene-corrected cells safer for clinical use.

WD disease phenotypes were not fully corrected after iHeps transplantation into mice livers. To achieve better partial therapeutic effects, we may need to obtain more mature/proliferative iHeps and increase iHeps in-vivo repopulation efficiency.

In conclusion, the present data demonstrated the capability of iHeps to serve as a potential cell source for autologous cell therapy for WD as well as other hepatic disorders. This proof-of-principle study provided evidence on utilizing stem cell-derived ex-vivo cell sources for in-vivo therapeutics.

Reference

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Kajiwara. Correction for Kajiwara et al., Donor-dependent variations in hepatic differentiation from human-induced pluripotent stem cells. Proceedings of the National Academy of Sciences 2012;109:14716-14716.

Contact information: (Email) weiruii@live.com; (Tel) 3917 9955