Commentary

Primary human hepatocyte gene editing: Prometheus' chains are loosening

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Primary human hepatocytes (PHHs) isolated from livers retain proliferative properties when xeno-transplanted into chimeric mouse models[1] or other species. Serial transplantation experiments in mice showed that PHH populations multiply by at least 100-fold per round,[2] illustrating their greater than million-fold expansion potential. This has sparked widespread research into therapeutic hepatocyte transplantation for patients with liver conditions. Two major roadblocks that have limited clinical progress with hepatocyte transplantation are the lack of renewable high-quality human hepatocyte sources and the inability to efficiently correct inborn errors in PHHs. In this issue of *Molecular Therapy*, Zabulica et al.[3] show the first genetic correction of an inborn error in PHHs.

The liver performs many metabolic and synthetic functions, including processing waste products, such as ammonia. It has a remarkable regenerative capacity that was featured in ancient mythology, perhaps most famously in Zeus' punishment of Prometheus. Most of the liver mass is composed of hepatocytes, which are spatially organized into lobules by blood vessels and bile ducts. After a partial liver resection, the majority of hepatocytes rapidly proliferate and restore the liver volume within 2 weeks. The cells that regenerate the liver in response to other types of liver injury are more diverse. Elegant cell tracing studies in mice have shown that several hepatocyte subsets and cholangiocytes that line the bile ducts can regenerate the liver parenchyma, depending on the type and severity of liver injury. Histopathological observations in human livers support these diverse repopulation mechanisms identified in rodents. As such, the liver harbors regenerative plasticity, with several cell types being able to replenish its parenchyma similar to observations in other organs.[4] Hepatocyte transplantation is being pursued as an alternative to whole organ liver transplantation, which would carry several advantages, including alleviation of donor organ shortages. In addition, hepatocyte transplantation has long held promise for autologous transplantation after genetic correction of inborn errors. The latter goal carries the added benefit that it would likely obviate the need for immunosuppression to prevent allograft rejection.

Both embryonic stem cells and, more recently, induced pluripotent stem cells can expand indefinitely. *In vitro* they can be subjected to efficient and reproducible differentiation protocols by which they acquire many hepatocyte functions.[5] These cells are commonly referred to as hepatocyte-like cells (HLCs) since they remain transcriptionally immature and functionally distinct from PHHs. In addition to their renewability, pluripotent stem cells can be efficiently edited using CRISPR-Cas9 to disrupt genes or, more recently, correct genetic variants.[6] Given these combined advantages, there have been widespread efforts to transplant HLCs into liver chimeric mouse models. Despite some encouraging reports showing engraftment, it has become increasingly clear that HLCs unreliably and non-reproducibly humanize liver chimeric animals. This starkly contrasts with the majority of PHH donors that reproducibly engraft and expand in these models. Since the engrafting cells in PHH populations remain undefined, mimicking robust PHH engraftment and expansion with HLC populations continues to be challenging.

Gene editing PHHs is being pursued as an alternative strategy to correct inborn errors in hepatocytes prior to therapeutic transplantation. There are a number of genetic conditions that cause liver and/or systemic diseases primarily through altered hepatocyte functions. These vary from rare conditions, such as urea cycle defects, to more common diseases, such as hemophilia B and alpha1-antitrypsin deficiency. CRISPR-Cas9 facilitates efficient and rapid gene editing in dividing cells, e.g. pluripotent stem cells. Because of the high editing efficiency, cells with the desired genotype can easily be selected and grown out to clonal populations. Editing non-dividing or terminally differentiated cells, such as PHH, has, however, remained much more challenging since non-edited cells cannot be easily removed.

Ornithine transcarbamylase (OTC) deficiency is a rare X-linked disorder of the urea cycle, resulting in ammonia elevation with life-threatening neurological complications. Zabulica et al.[3] identified a splice acceptor site variant that results in an early stop codon and dysfunctional protein in an OTC patient who underwent liver transplantation. Using synthetic guide RNA and recombinant Cas9 ribonucleoprotein complex transfection, the authors were able to remove the splice variant in 60%–80% of PHHs isolated from the explanted liver and restore OTC function. They then exploited the proliferative potential of PHHs and transplanted genetically corrected hepatocytes from this OTC patient into liver chimeric mice. This resulted in restored ammonia metabolism *in vivo*. These remarkable results show that CRISPR-Cas9-edited PHHs retain the ability to expand in liver chimeric mice and that partial genetic correction of OTC is sufficient to cure the condition in laboratory animals. These findings will be useful immediately to study OTC deficiency in human hepatocyte models and accelerate therapeutic

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PHH transplantation efforts for this condition.

The technical advances by Zabulica et al.[3] will also be a major impetus to correct other genetic variants in PHHs. Nonetheless, several challenges remain. Most inborn errors will require correction,[6] rather than deletion, which, to date, remains inefficient in non-dividing cells that cannot be selected for clonal populations. And, while the approach by Zabulica et al.[3] worked well with PHHs from an OTC patient, generalizability to other liver diseases that cause inflammation and fibrosis will be more challenging. PHHs isolated from inflamed or cirrhotic livers generally fail to repopulate liver chimeric models. For those conditions, alternative strategies need to be developed to gene edit and replenish the liver parenchyma, e.g., by harnessing the plasticity of cholangiocyte organoids that can be isolated from cirrhotic livers and recently were shown to engraft in human bile ducts.[7] Thus, after having been chained to a rock for thousands of years, several technological breakthroughs are starting to unleash the liver's regenerative potential. These advances may soon begin to benefit patients with liver conditions.

REFERENCES

1. Grompe, M., and Strom, S. (2013). Mice with human livers. Gastroenterology *145*, 1209–1214.

2. Michailidis, E., Vercauteren, K., Mancio-Silva, L., Andrus, L., Jahan, C., Ricardo-Lax, I., Zou, C., Kabbani, M., Park, P., Quirk, C., et al. (2020). Expansion, in vivo-ex vivo cycling, and genetic manipulation of primary human hepatocytes. Proc. Natl. Acad. Sci. USA *117*, 1678–1688.

3. Zabulica, M., Srinivasan, R.C., Akcakaya, P., Allegri, G., Bestas, B., Firth, M., Hammarstedt, C., Jakobsson, T., Jakobsson, T., Ellis, E., et al. (2021). Correction of a urea cycle defect after ex vivo gene editing of human hepatocytes. Mol. Ther. *29*, this issue, 1903–1917.

4. Shivdasani, R.A., Clevers, H., and de Sauvage, F.J. (2021). Tissue regeneration: Reserve or reverse? Science *371*, 784–786.

5. Heslop, J.A., and Duncan, S.A. (2019). The Use of Human Pluripotent Stem Cells for Modeling Liver Development and Disease. Hepatology *69*, 1306–1316.

6. Anzalone, A.V., Randolph, P.B., Davis, J.R., Sousa, A.A., Koblan, L.W., Levy, J.M., Chen, P.J., Wilson, C., Newby, G.A., Raguram, A., and Liu, D.R. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. Nature *576*, 149–157.

7. Sampaziotis, F., Muraro, D., Tysoe, O.C., Sawiak, S., Beach, T.E., Godfrey, E.M., Upponi, S.S., Brevini, T., Wesley, B.T., Garcia-Bernardo, J., et al. (2021). Cholangiocyte organoids can repair bile ducts after transplantation in the human liver. Science *371*, 839–846.