Hepatocyte transplantation, a step forward?

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Hepatocyte transplantation is still to consolidate as a viable therapeutic option for patients with hereditary metabolic liver diseases. Although we now oversee five decades of basic and animal research in the field, the number of successfully treated patients remains low. However, the discrepancy between successful cell transplantation in animals and the often marginal therapeutic outcomes in patients is striking. Animal experiments are optimally designed to achieve the best possible outcomes, and the use of animal models that enable a selection growth advantage for transplanted cells (i.e., freshly isolated, high quality autologous hepatocytes) can serve as an example for a mismatch between basic science experimental scenarios with the clinical reality, which relies on heterologous hepatocytes isolated from marginal liver donor organs. Attempts to substitute primary isolated heterologous hepatocytes with autologous iPS cell derived hepatocytes have remained elusive and still await conclusive results for its broader clinical applicability.

In this issue, Barahman, Guha and colleagues have studied the use of focal radiation combined with hepatic cell growth stimulation, as a clinically applicable therapeutic modality to increase engraftment and repopulation of a host liver with transplanted hepatocytes (**1**). The apolipoprotein E “knock out receptor deficient mouse model”, which they have used, does not provide a selection advantage for transplanted cells and has thus rarely been used in earlier studies. The therapeutic outcome was analyzed not only histochemically by calculating the number / percentage of successfully engrafted cells, but also monitored by increased ApoE levels and reduced cholesterol, LDL, VLDL levels in serum. Infusion of hepatocytes via portal

vein or into the spleen usually results in less than two percent of engrafted cells and would not significantly change lipid serum levels in this mouse model. The authors of the study explored a strategy based on regional high dose irradiation of mice liver combined with hepatic cell growth stimulation and analyzed the effects on engraftment and functionality of hepatocyte transplantation. Previous studies of the Guha group and others had already shown a dose dependent inhibitory effect on proliferation of liver hepatocytes with maintenance of liver function and only a slow clearance of the irradiated cells (**2**). Radiation therapy (50 Gy) was restricted to median and right lobes of the mouse liver. Although this focal radiation of the host liver may diminish the therapeutic effects of transplanted cells, in turn, it reduces the risk of liver failure in unsuccessful cell transplants.

With this approach the number of transplanted cells was significantly, but not impressively increased, as compared to control animals (transplanted without irradiation). Initially engrafted hepatocytes would probably increase their number over a long period of time, since the irradiated host hepatocytes slowly die after the previous high dose irradiation. To accelerate the process of liver repopulation, Guha et al used growth stimulators such as adenovirus encoded hepatocyte growth factor (HGF), or GC1, a selective agonist of the T3 thyroid hormone β receptor, which elicits a strong hepatic mitogen stimuli but has no impact on cardiac function as thyroid hormones have. Four month after hepatocyte transplantation the number of engrafted hepatocytes had dramatically increased and serum cholesterol, LDL and VLDL levels reduced to almost normal levels. Based on these evidences, the authors conclude that focal radiation in combination with a hepatic growth stimuli improves the therapeutic outcome of hepatocyte transplantation and successfully correct this hereditary liver disease in the absence of a selection advantage for transplanted cells. Side effects were acceptable with no long term safety concerns, although these issues were not in the focus of the study.

Preparative radiation in the context of hepatocyte transplantation was already explored in a pioneer work by Soltys et al., in a small number of non-human primates, as a basis for the *first-in-man* trial, and published in this journal (**3**). Three patients with hereditary liver diseases were transplanted after prior focal irradiation of the liver. The results evidenced the clear positive effects of previous liver irradiation, although detailed data on long term outcomes and safety issues were not reported.

The study of Guha group convincingly shows the potential of radiation therapy combined with hepatocyte growth stimuli to achieve an improved engraftment of transplanted hepatocytes, even if there is not a selective advantage for them, and an efficient therapeutic correction of an inborn error of the liver. The work provides detailed analysis on a cellular and molecular level and gives support for further clinical development. Whether the administration of HGF or the agonist GC1 is safe and efficient in humans, remains to be determined. Furthermore, few data currently exist on long term safety of radiation preconditioning of the liver, especially in children. Although no long term consequences have been reported in one retrospective study (**4**), careful monitoring of patients treated with radiation therapy and HT is mandatory.

Partial hepatectomy has been used as a clinical procedure to increase the number of successfully engrafted hepatocytes, and has been applied in two patients with Crigler-Najjar syndrome, but it did not result in complete correction of the disease (**5**). Other alternative therapeutic approaches to correct hereditary metabolic liver diseases are being currently explored. Adeno-associated virus (AAV) used in clinical gene therapy protocols (i.e., hemophilia B) with promising results (**6**), are being explored in liver diseases as well (**7**). Whether this type of gene therapy can be successfully applied to newborns and small children remains to be verified, since episomal AAV vectors may be lost in a growing liver. More recent studies point at the potential of CRISPR/Cas9 based HDR and base editing methods for in vivo correction of genetic diseases of the liver (**8**).

Currently, we cannot anticipate, which of these advanced therapies will be the one of choice to correct hereditary metabolic liver disease in a near future, but Chandan Guha and co-workers **(1)** have clearly shown that hepatocyte transplantation together with focal radiation and growth stimuli may be one promising candidate.

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