Mice with humanized liver endothelium

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Summary

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The liver is one of the largest organs in the human body and plays an important role in various metabolic functions. Thus far, the only curative treatment option for a large proportion of patients suffering from a liver disorder is liver transplantation. This is however a very invasive procedure and, due to shortage of healthy donor livers, only limited numbers of patients can benefit from this procedure. The use of *ex vivo* genetically modified autologous lever cells could overcome the problem of donor scarcity. For several years now, scientists have therefore studied the potential of cell transplantation, instead of whole liver transplantation, as a possible treatment option. Even though clinical trials have shown that transplantation of liver cells is feasible, long-term outcome is disappointing. Other obstacles in clinical implementation of hepatocyte transplantation such as poor engraftment of transplanted donor cells and difficulty of manipulating hepatocytes *in vitro* also remain. These considerations highlight the need for development of better transplantation procedures.

Poor translation of animal studies to humans is one of the reasons for the disappointing outcome of clinical studies on cell transplantation. In the present thesis, we sought out to solve this problem by developing a mouse with a "humanized liver", which would serve as an excellent *in vivo* model for studies on liver-directed cell and gene therapy. A mouse with a humanized liver can be developed by transplanting human liver cells into the spleen of immune deficient mice. In the first set of experiments performed for this thesis, we found that human fetal liver sinusoidal endothelial cells were able to engraft and repopulate the mouse liver. This serendipitous finding set a basis for subsequent experiments with the aim to:

1) Identify cells suitable for liver engraftment
2) Use mice with humanized liver endothelium in gene therapy

In **chapter 1** an overview of the current knowledge about the normal anatomy, physiology and development of the liver is given. These include embryonic liver hematopoiesis, liver regeneration and characteristic features of the liver that are important for understanding the experiments described in this thesis. Additionally, the importance of developing treatment options for patients suffering from a liver disorder is emphasized and the most recent developments in liver-directed cell

and gene therapy are reviewed. Endothelial cells form the inner lining of blood vessels and are present throughout the human body. In this chapter we also describe the heterogeneity and origins of endothelium.

In **chapter 2** we describe transplantation of human liver sinusoidal endothelial cells in the spleen of immune deficient mice. The endothelial cells were transduced with a GFP-expressing lentiviral vector prior to transplantation to facilitate tracking. Following transplantation, human liver sinusoidal endothelial cells engrafted and repopulated throughout the mouse liver. We also investigated the capacity of other types of endothelial cells to engraft and repopulate liver by transplanting human macrovascular and microvascular endothelial cells in the spleen of immune deficient mice. In contrast to human liver sinusoidal endothelial cells, human macrovascular and microvascular endothelial cells were not able to engraft the mouse liver. In this chapter we show that only human liver sinusoidal endothelial cells have the unique capacity to engraft and repopulate the mouse liver niche, indicating that mature endothelial cells cannot transdifferentiate *in vivo* and thus do not exhibit phenotypic plasticity. The results from the experiments found in this chapter set a basis for further research to the potential of human liver sinusoidal endothelial cells in liver-directed cell and gene therapy as described in following research chapters.

The presence of recipient endothelial cells in donor liver biopsies several months after liver transplantation raised the possibility that circulating bone marrow-derived progenitor cells contribute to repair of damaged liver endothelium. Like bone marrow, the human fetal liver is a rich source of progenitor cells. In **chapter 3** we examined whether hematopoietic progenitor cells from the human fetal liver have the capacity to differentiate into endothelial cells *in vivo.* We used two different approaches to try and answer this question. Hematopoietic progenitor cells (HPC) from human fetal liver were transplanted in immune deficient mice to reconstitute a human immune system. Subsequently, the liver endothelium was damaged and repair by human hematopoietic cells examined. In the second approach, hematopoietic progenitor cells from human fetal liver were directly transplanted in immune deficient mice with damaged liver endothelium and repair by human cells was also examined. In neither of these conditions restoration of mouse liver endothelium with human cells was observed, indicating that human fetal liver-derived hematopoietic progenitor cells are not a source of liver

endothelium and that repair of damaged liver endothelium mainly occurs through outgrowth of differentiated (non-)circulating endothelial cells.
For many disorders, clinical implementation of *ex vivo gene* therapy will require the ability to regulate expression of genes to maintain expression levels within a therapeutic window. The aim of **chapter 4** was to investigate, which human fetal liver cell type can be most efficiently transplanted and used for regulated *ex vivo* gene therapy. After transplanting human fetal liver cells in immune deficient mice, no engraftment of human hepatocytes could be detected. However, following transplantation of unsorted human fetal liver cells, abundant reconstitution of murine liver with human endothelium was achieved. Furthermore, following *in vitro* transduction using an auto-regulatory lentiviral vector, transplanted human liver sinusoidal endothelial cells were able to express erythropoietin *in vivo* only in the presence of doxycycline. These findings demonstrate that human liver sinusoidal endothelial cells are a promising cell type for long-term *ex vivo* regulated gene therapy.

The potential of our mouse model for studying vector targeting is described in **chapter 5.** We show that upon systemic administration of a human CD105 targeted lentiviral vector, human liver endothelial cells in mice with humanized liver endothelium were specifically transduced. These results demonstrate that our mouse model with a humanized liver endothelium is very useful for the evaluation of targeted *in vivo* gene transfer.

The results of the experiments described in this thesis have set a basis for the use of human liver endothelial cells in cell transplantation and *ex vivo* gene therapy. In the general discussion **chapter 6**, the data of our work are discussed in relation to recent developments in the field of cell and gene therapy A lot of challenges remain, but hopefully the progress that has been made in the field of cell transplantation and *ex vivo* gene therapy so far will lead to potential treatment options for patients that suffer from a liver disorder.