

## **LIVER REPOPULATION STRATEGIES**

Guidotti J.E., Mitchell C., Mallet V.O., Kahn A., Gilgenkrantz H.

*Department of Genetics Development and Molecular Pathology, Cochin Institute, Paris, France*

### **Liver regeneration: a new model for an old trick!**

Liver has kept the extraordinary capacity to spontaneously regenerate after an injury as it has been illustrated a long time ago with Prometheus myth! This capacity of the adult human liver to regenerate is still used nowadays in large surgical resections, in living donor transplantations or in “split liver” procedures. Although highly differentiated and quiescent cells, hepatocytes do orchestrate liver regeneration. It has been mainly documented after two third hepatectomy in rodents. We have created a new inducible model of liver regeneration based on a physiopathological signalling pathway involved in liver disorders of various origins, apoptosis. These transgenic mice express a modified chimeric caspase-3 activated only upon injection of a non-toxic chemical inducer of dimerization specifically in the hepatocytes. The liver of these animals is normal in the absence of induction. Upon induction, dimerization of caspase-3 leads to its activation and apoptosis proceeds in the hepatocytes in a dose-dependent manner. Although 80% of hepatocytes are destroyed by this cell death program in a few hours after induction, an intensive regenerative process will allow the 20% remaining hepatocytes to proliferate and reconstitute a normal hepatocytic mass. The liver completely recovers in 10 days. This strategy of a specific cell-type ablation provides a pertinent model to study liver regeneration [1].

### **The concept of liver repopulation: hepatocyte as a stem cell!**

Liver transplantation is the only available therapy for end-stage liver failure. However, shortage of donor livers hampers orthotopic liver transplantation worldwide and about 10 to 15% of these patients will die while waiting for liver transplantation. Given the proliferative capacity of adult hepatocytes, a cell-based therapy for liver regeneration has been proposed as an alternative promising approach. However, the potential of this strategy is challenged by *ex vivo* hepatocyte viability, poor cryopreservation and most importantly by the low proportion of cells that engrafts and maintains in the transplanted liver. Consequently, only a few clinical trials have been reported to show a therapeutic benefit in liver metabolic disorders such as Crigler Najjar, Wilson’s disease or glycogen storage diseases.

The use of a viral vector *in vivo* has been proposed as an alternative therapeutic strategy to overcome this low efficiency of hepatocyte transplantation. However, up to now,

none of the classical used vectors led to both effective and prolonged liver transduction directly *in vivo*. In contrast, an efficient and rapid transduction of human hepatocytes can be obtained *ex vivo* using lentiviral vectors [2]. A third concept of liver targeted gene therapy has recently emerged, combining the advantages of both approaches. This strategy is based on the capacity to confer a selective survival advantage of transplanted hepatocytes compared to resident ones. This selective survival advantage has been brought in overexpressing the anti-apoptotic Bcl-2 or Bcl-xL proteins into transplanted hepatocytes. Hepatocytes overexpressing these anti-apoptotic Bcl-2 family members are indeed protected against a apoptotic challenge mediated by Fas pathway. Upon transplantation in the liver of animals submitted to repeated Fas-dependent apoptotic challenges at non-lethal doses, Bcl-2 or Bcl-xL expressing hepatocytes selectively proliferate and progressively repopulate the liver of the recipients [3,4]. This selective survival advantage conferred to hepatocytes that represent less than 1% of the recipient's hepatocytes before apoptotic challenges, allow them to reach more than 80% of the mouse liver parenchymal cells in about 2 months, underlining the powerfulness of this strategy [5]. To demonstrate the therapeutic value of this strategy, we repeated the procedure on Apolipoprotein E deficient mice. These mice show a severe hypercholesterolemia and arterial atherosclerosis upon normal regimen. Transplantation of Bcl-2 expressing hepatocytes in mutant animals induced a significant lowering of total cholesterolemia and a clear diminution of atherosclerosis suggesting not only that this approach is therapeutic but that repopulating hepatocytes are indeed functional [6].

There are theoretically two ways to confer a selective advantage to hepatocytes : one can give them either a survival advantage (see Bcl-2/Fas or BclxL/Fas models), or a proliferative advantage. To test this latter approach, we have transplanted hepatocytes overexpressing FoxM1B (kindly givent by R. Costa, Chicago). FoxM1-B is a transcription factor expressed in proliferating organs. It is not expressed in the quiescent liver but it is activated during liver regeneration process. Its overexpression is not tumorigenic but will induce an eight hours acceleration in the onset of hepatocyte DNA replication during liver regeneration. Interestingly, we found that, compared with control hepatocytes, FoxM1B overexpressing hepatocytes repopulate better livers induced to chronic and continuous process of injury and regeneration.

### **Another adult stem cell compartment for regenerative liver medicine : a myth?**

Are there other cell-types more easy to handle to assume the hepatocyte function? In other words does a stem-cell niche exists within or outside the adult liver? It is only when

hepatocytes are hampered to proliferate that « stem-like cells » usually called oval cells, localised in a specific niche, can function as bipotent progenitor cells giving rise to hepatocytes and cholangiocytes. However, it has not been possible until now to purify to homogeneity this discrete and heterogeneous population. Moreover, these cells have been involved in the development of cholangiocarcinomas.

Recently, numerous studies have highlighted the potential of adult bone marrow cells, more easy to handle than liver cells, to differentiate into various cell types including hepatocytes. However, the differentiation efficiency of marrow cells into hepatocytes *in vivo* and its importance in physiopathological processes is still debated.

We have studied the ability of bone marrow cells to participate to liver regeneration using our Bcl2/Fas model of liver repopulation. Because in transgenic animals the Bcl-2 transgene is under the control of a liver-specific promoter, Bcl-2 expression in the liver after bone marrow transplantation would attest for the differentiation of bone marrow cells into hepatocytes. We transplanted lethally irradiated wild type female mice with bone marrow from Bcl-2 transgenic males, and submitted the animals to Fas-induced apoptosis. We showed that, under physiological circumstances, differentiation frequency of bone marrow cells into mature hepatocytes is under  $1/10^5$ , therefore far below any therapeutic level. We also demonstrated that, in the absence of irradiation, the presence of Bcl-2 expressing cells could not be detected, underlining the requirement of a bone-marrow reconstitution to detect this phenomenon [7]. When 30% of the liver was reconstituted with hepatocytes after 2 months of Fas selective pressure, less than 1% was obtained with bone marrow stem cells after lethal irradiation of the animals in the same period of time!

This paucity of bone-marrow derived hepatocytes has been described in other models and several experimental data explained this event by a fusion between a bone-marrow derived donor myelomonocytic progenitor and a resident hepatocyte [8-9]. The differences in the types of donor cell subpopulations studied, in the methods of detection of donor-derived cells and in the recipient models used hampered finally to conclude about the real plasticity of these cells. Moreover, in the light of recent discoveries, one could also think that, in some of these experiments, bone marrow-derived hepatocytes could have originated from the mesenchymal bone marrow compartment and not from the hematopoietic one. For two years, multipotent adult progenitor cells (MAPCs) described by C. Verfaillie's group were the only documented post-natal bone marrow cell population capable at a clonal level of *in vitro* differentiation into hepatocytes making them an extremely promising candidate for liver cell therapy [8]. However, their therapeutic potential has never demonstrated and particularly their

level of participation to liver repopulation still awaits confirmation. More recently, other cell types have been isolated and shown to be capable of differentiating into various cell types including liver cells either *in vitro* or *in vivo* [10-13]. It will be now important to compare side by side these categories of cells for their availability and their efficiency to participate to liver regeneration and to function without inducing tumorigenicity.

Finding a universal multipotent postnatal stem cell that could help to bridge patients to liver transplantation, provide metabolic support during liver failure or replace hepatocyte mass in metabolic liver-diseases cell sounds like the Holy Grail quest. However, recent data seem to converge in the notion that the hepatocyte still remains the most highly efficient « stem cell » for the liver. We have contributed to demonstrate that a selective advantage conferred to these hepatocytes can dramatically enhance the proportion of these therapeutic cells allowing them to reach a therapeutic purpose. However, genes that could prove useful as targets for liver repopulation in clinical trials remain to be found.

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