TRANSLATION FROM MICE TO MEN: ARE DOGS A DODGY INTERMEDIATE?

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Disclosure: No potential conflict of interest.
Received: 04.02.14 **Accepted:** 09.04.14
Citation: EMJ Hepatol. 2014;1:48-54.

ABSTRACT

Alternatives for liver transplantation in severe liver disease are urgently needed in view of the limited availability of donor livers. The use of embryonic stem cells (ES) or hepatic progenitor cells (HPC) has been investigated in mice models of acute and chronic liver failure. To extrapolate the findings in inbred mouse strains (weighing around 20 g, with a maximal lifespan of 3 years) to the genetically more variable human beings (around 3,000-fold heavier and living 30 times longer), does seem a bit of a large step. This article describes recent developments in HPC research in dogs and compares these findings to experimental rodent studies and human pathology. Recent progress in canine liver stem cell research and canine genetics are combined to exemplify their possible role as a relevant animal model for the feasibility of stem cell transplantation in human liver failure.

Keywords: Liver, progenitor cell, cell transplantation, inherited diseases.

INTRODUCTION

The limited availability of donor livers is one of the drawbacks in liver transplantation. Regenerative medicine, not only in hepatology, is at a crossroads between fundamental research and the clinical application of either stem/progenitor cell or differentiated cell (for liver research, the hepatocytes) transplantations.[1] Where transplantation of fully differentiated hepatocytes seems obvious for parenchymal diseases, their application in biliary diseases seems of little meaning. In contrast, hepatic progenitor cells (HPCs) have the potential to differentiate into both hepatocytes and cholangiocytes, offering a potential treatment modality for parenchymal and biliary diseases. The presence of HPCs has been under dispute for about five decades, ever since the first description by Farber in 1956.[2] The necessity of these cells seems limited since fully differentiated hepatocytes and cholangiocytes can, in contrast to most other differentiated cells, proliferate.[3] This is exactly what they do in acute liver failure or after partial hepatectomy. However, in those circumstances where their replication is hampered, HPCs come into play in an effort to repopulate the affected liver. HPCs reside in the Canals of Hering, where they are in close proximity with stellate cells, Kupffer cells, together constituting the HPC-niche. Histologically, HPC activation in a diseased liver section is described as 'ductular reaction' or 'bile duct proliferation'.[3] This indicates that activation of HPC could be beneficial for the liver to recover upon injury. At the same time, there is a potential down-side of activated HPC. The two-faced (Janus-like) character of HPCs is shown by the presence of progenitor cell markers in hepatocellular carcinoma (HCC), indicative for malignancy in humans and dogs.[4,5] Since potential risks and lack of data from hepatocyte transplantations are the most common restriction for participating in a Phase I hepatocyte transplant trial, it is clear that animal models predicting long-term risk/benefits are urgently needed.[6] In this paper, the predictive potential of dogs in cell transplantation in diseased livers is addressed.

Requirements for Animal Models

Important requirements for a good animal model in liver cell transplantation studies include: firstly, phenotypic resemblance with the human clinical situation, and secondly, good experimental controllability. The phenotypic resemblance is evaluated below with emphasis on HPCs and their activation differences in liver diseases in both men and dogs. The controllable, and thus reproducible, experimental set-up is described in view of the specific population structure in different dog breeds.

THE HPCS

HPCs are present as quiescent cells in healthy adult liver tissue in small numbers in the Canals of Hering, the smallest ramifications of the intrahepatic biliary tree. Located close to the portal area they are at the interface between hepatocytes and cholangiocytes.[7] Although simply stated as HPCs, their cellular origin remains an area of debate, let alone the question of whether one or more HPC pools do exist. A number of studies state a possible biliary origin of HPCs, whereas other studies in humans describe extrahepatic peribiliary glands as the prime location for HPCs, and the haematopoietic origin of HPCs is also suggested several times.[8-17] For this review, we focus on the HPC-niche within the Canals of Hering, as described in numerous mammals.[3,18-21]

HPCs can be histologically characterised by a combination of their specific morphology upon activation (the so-called ductular reaction) and by non-cell specific marker expression.[7] The classical HPC-markers, including the cytokeratins keratin-7 (K7) and keratin-19 (K19), are expressed on cholangiocytes too, which underpins the necessity to combine marker expression with histology. The same non-HPC specificity holds true for other stem cell markers including CD133 (PROM1) and EpCAM, which are for instance expressed on other stem cells.[22] In view of the versatile character of HPCs it is no surprise that mesenchymal markers such as CD29 (integrin β1) and CD44 (hyaluronic acid receptor and co-receptor for hepatocyte growth factor) are expressed on HPCs. For an extended list including other species see Kruitwagen et al.[23] For only a subset of markers, for instance ABCG2, CD44, CD133, K7, and K19, the expression is measured in all three species.[20,24-26]

HPC Activation in Liver Diseases

Just a few publications describe the HPCs and the HPC-niche in dogs, and make a comparison with either mice and/or humans.[27,28] This comparison is mainly based on immunohistochemical analyses. In contrast to humans, in mice and dogs the availability of healthy liver samples allows for a diseased-healthy comparison. It must be taken into account that the aetiology of human liver diseases is often different from the experimental mouse models. Virus-induced hepatitis is difficult to induce experimentally, but, as outlined below, hepatic copper toxicosis can be observed in all three species. The location and characteristics of quiescent canine HPCs and portal myofibroblasts were characterised in healthy livers. HPCs were located in the space of Disse, as previously described for other species.[29] A descriptive immunohistochemical study evaluated the inflammatory infiltrate and fibrosis in samples of canine chronic hepatitis. A positive correlation was found between the stage of fibrosis and the number of myofibroblasts (alpha-SMA positivity) and bile duct proliferation.[30]

Another study reported a positive correlation between tenascin-C expression, a specific component of the extracellular matrix (ECM), and stage of fibrosis, degree of inflammation, and the number of K7 positive cells in canine chronic hepatitis.[31] These findings extend the knowledge derived from murine and human samples to the canine HPC-niche and its activation during severe liver disease. For more direct human-dog comparisons, the relation between HPCs, stellate cells, fibrosis, and disease severity in healthy and diseased livers, was described in liver samples from both species. In liver disease with fibrosis, HPC activation was most pronounced and activated stellate cells were in close proximity to the ductular reaction.[32,33] Suggestive for having a crucial role of ECM, the component laminin co-localised with activated stellate cells and HPCs and macrophages clustered at the site of injury, more specifically periportally in acute hepatitis and in the fibrotic septa in chronic hepatitis [unpublished data].

HPCs in Regenerative Medicine

Having established, as summarised from marker expression in Table 1, that HPC-activation in rodents, dogs, and men is highly similar, both at the histological and at the molecular level, the

Table 1: Markers used to investigate mouse, dog or human hepatic progenitor cells. Due to space limitations, reference to all original papers has not been possible, therefore occasionally only reviews are referred to.

Marker	Mouse	Dog	Human
A6	82, 83		
ABCG2/BCRP1	84	20	20, 34
AFP		88	34, 89, 90, 91
Albumin	85		90, 91, 92
DLK	86		
c-Kit			34, 91
CD 24	83		
CD29		88	89
CD44	36	88	34, 90, 91
CD73			89
CD90			89
CD133	36, 85, 86, 87	88	34
CLDN3			90
Chromogarnin-A			90, 93
EpCAM	83, 85, 86		94, 95
FN14/TWEAK-R	86	88	
HNF4-alpha		88	
ICAM1			90
Keratin-7	9, 85	28, 20, 88	20, 28, 34, 35, 38, 94, 95, 96
Keratin-8			90, 95
Keratin-18			90, 95
Keratin-19	79, 10, 85, 86	28, 88	28, 34, 35, 38, 91, 92, 94, 95, 96
Lgr5	36		
NCAM			34, 90, 94
OPN	10	88	
OV6			94, 95
Sca1	86		
Sox9	9, 10, 36, 86, 87	88	
Vimentin			89

For an extended list with more markers and more mammals including rats and cats see Kruitwagen et al.,[97] from which this table is adapted.

question arises of how to implement these findings into a canine model of HPC transplantation for the benefit of human clinical practice.

Autologous versus allogenic and *ex vivo* culture versus *in vivo* stimulation

In my opinion, technically it is possible to harvest autologous HPCs, expand them in culture and differentiate them into hepatocytes for transplantation purposes. This process is most likely too time-consuming for acute liver failure. In the case of inherited metabolic disease, gene correction could be applied before transplantation. Healthy dog livers contain a 'side population' enriched in progenitor cells, and canine HPCs can be cultured *in vitro* upon isolation from healthy liver tissue.[34,35]

Using a plate-and-wait method, colonies of canine HPCs grew from the non-parenchymal fraction of a digested liver sample within a few weeks. As stated above, in cases of urgent clinical need, this culture method as an autologous source for transplantation would not be feasible. In chronic cases, however, this would be an option and would circumvent rejection issues. Optimisation of culture conditions of primary HPCs is needed in addition to characterisation of cells in culture, most importantly, self-renewal and differentiation capacity and stability. A promising recent development, more specific than 'side population' or plate-and-wait, is the discovery of the Wnt-driven stem cell marker Lgr5 positive cells in injured mouse livers that can be fluorescence-activated cell sorted (FACS) or isolated as 'ducts' and form organoids upon 3D culturing.[36] These cells rapidly expand, have the capacity to differentiate into hepatocytes, and can be kept in culture for more than a year, while maintaining their genomic integrity. The existence of canine liver organoids needs to be established.

More challenging is the *in vivo* stimulation of HPCs. For this, it is of utmost importance to unravel the molecular pathways involved in HPC activation (proliferation, migration, differentiation). This has been extensively studied in rodent models, and to a lesser extent, in human and canine samples.[37-44] Amongst the activation signalling pathways are the well-known stem cell regulators such as Wnt/beta-catenin and Notch signalling. Since activating mutations in these pathways leads to various forms of cancer, it is obvious that long-term follow-up of interference in these pathways is needed before its application in the human clinical setting.

Liver Tumours

There is an obvious association between HPCs and liver tumours, both in man and dog. This association is plausible as HPCs have self-renewal capacity and migratory potential, which is required for invasion and metastasis.[45] HPCs are described as a possible cell of origin for HCC, although this lineage relationship is not directly proven.[4,46-49] Alternatively, the presence of HPC markers in HCC is in line with the possible de-differentiation of fully matured hepatocytes undergoing malignant transformation, and subsequently the expression of immature markers such as K19 in HCCs.[44,50] There is overwhelming clinical evidence that expression of HPC markers, especially K19, in human HCC is a negative prognostic indicator, as these tumours show a higher recurrence rate and shortened patient survival.[4,50] In dogs, the presence of progenitor (K19) and malignancy (glypican-3) markers was evaluated immunohistochemically; the occurrence of K19 positive HCCs was 12%, which resembles the prevalence in humans.[5] Whether, in line with the stem cell marker expression in HCC, men and dogs are similar regarding Wnt and Notch signalling in HCC remains to be answered.

HOW TO PROCEED?

With respect to HPC transplantation, metabolic diseases will probably be the first to be addressed in dogs. Transplantation of hepatocytes has been reported in a few studies of Dalmatians as a model for hyperuricosuria.[51-53] In order to standardise the experimental conditions as much as possible, a large or mid-sized animal model with a well-defined and simple inheritable disease, and a clear phenotype, is ideally suited to evaluate route of cell transplantation (e.g. portal vein versus hepatic artery), to measure short-term transplant engraftment and restoration of liver function. The lifespan allows investigation of long-term effects including the potential risk of tumour formation initiated by the transplanted cells.

In the mid-seventies, a progressive form of chronic hepatitis, accompanied by high levels of liver copper, was first described in the Bedlington Terrier in the United States.[54] It took almost three decades before the responsible gene was identified by means of positional cloning.[55]

A genomic deletion of 39.7 kb covering exon 2 of the *COMMD1* (the gene formerly known as *MURR1*) gene caused a complete absence of the protein product, leading to extreme accumulation of hepatic copper.[55,56] Gene silencing and *COMMD1* -/- mice and dogs confirmed its role in hepatic copper accumulation.[57-60] *COMMD1* is ubiquitously expressed and is involved in many cellular functions including sodium metabolism, regulation of NFκB, and HIF-1alpha-mediated transcription.[57-68] The common denominator in these processes is the fact that ubiquitylation of these proteins is mediated by *COMMD1*. Recent data indicate that *COMMD1* plays a role in the functioning and stability of the human Wilson's disease gene *ATP7B*,[1] providing a clue to how *COMMD1* absence leads to copper accumulation within hepatocytes.[69]

A *COMMD1*-deficient dog presenting with copper storage disease resulting in chronic hepatitis,

provides an excellent model for clinical HPC transplantation trials in view of the requirement for a suitable animal model. It is genetically well-defined hepatitis and fibrosis progression that have been described in detail, and this metabolic disease resembles Wilson's disease. Diseases in a more advanced stage including cirrhosis and ECM remodelling will be more challenging. These types of diseases will require a multi-modular strategy targeting hepatocyte regeneration, fibrosis resolution, and modulation of inflammation. Current developments in anti-fibrotic therapies and the co-transplantation of mesenchymal stem cells (MSCs) or macrophages to modulate inflammatory responses are promising but are currently at the *in vitro* and rodent level.[70,71]

CONCLUSION

There is much promise in the use of HPCs in regenerative therapies for human medicine. In dogs, important molecular and cellular reaction patterns in particular liver diseases are reported, and characterise HPCs and their niche. Overall, HPC marker expression in dogs is comparable to that of humans, as is response to injury and the cell types involved in modulating HPC response. This suggests that the therapeutic potential of these cells is similar in dogs when compared to man, and opens up the potential for developing new strategies for currently untreatable liver diseases, positioning dogs as potentially important animal models to progress from bench-to-bedside.

Yet, this might just be the beginning of the (re-)appreciation of dogs in regenerative and translational medicine. The discovery of the genetic background of hepatic copper accumulation in Bedlington Terriers is an example of simple Mendelian recessive inheritance. Complex human genetic disorders are much more difficult to investigate; cohorts of thousands of participants are needed here. For instance, the phenotypic variation in human Wilson's disease patients and the genetic background of Endemic Tyrolean Infantile Cirrhosis (ETIC), Indian Childhood Cirrhosis (ICC), and idiopathic copper toxicosis are unexplained, partially due to low patient numbers and small pedigrees. Dogs have an ideal population structure for exploring the genetic basis of a variety of disorders, both Mendelian and complex.[72] As a consequence of inbreeding, the genetic complexity of these diseases is reduced. Therefore, inbred dogs are a genetic magnifier, instrumental to discovering crucial and modifier genes involved in Mendelian and complex genetic diseases in humans.

Some examples of complex genetic liver disorders in dogs include copper-associated hepatitis in the Labrador Retriever, Dobermann, West Highland White Terrier, and Dalmatian.[73-77] In these breeds a complex form of copper-associated hepatitis is present, where the susceptibility for copper is genetically determined and the expression of the disease phenotype (severity and/or time of onset) relies on environmental factors like dietary copper intake. Another example is congenital portosystemic shunting, which is a very rare disease in humans, but a much more frequently observed disease in several dog breeds such as Irish Wolfhounds, Labrador Retrievers, or Cairn Terriers.[78-80] Identification of the genetic components involved in this disease will not only be useful for those patients suffering from a congenital portosystemic shunt but may have broad implications for hepatic angiogenesis in general.

The potential benefits of including dogs as an intermediate between rodent studies and human clinical practice does, in fact, close the liver transplantation cycle. It was in 1961 that Starzl and colleagues[81] reported for the first time on liver transplantation in dogs; the field of liver transplantation benefited greatly from this landmark work by Starzl and colleagues.[81] Dogs might also prove to be useful in the next 50 years, and not just a dodgy intermediate.

Acknowledgements

HS Kruitwagen and BA Schotanus received funding from the Netherlands Science Foundation (NWO-ZonMW) for translational research on hepatic progenitor cells.

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