^{31}P Magnetic Resonance Spectroscopic Imaging of Liver Repopulation by Transplanted Hepatocytes

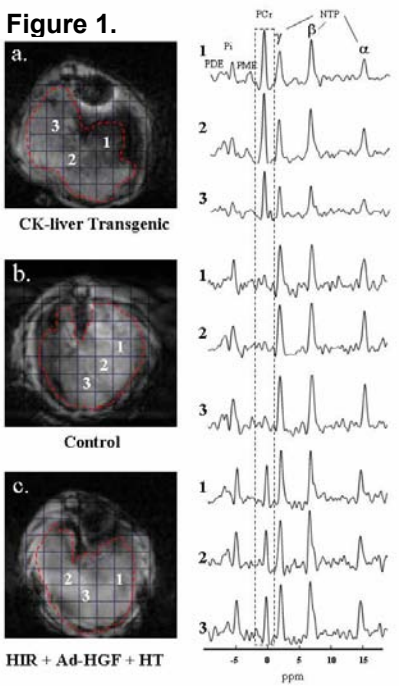
C. Landis[1], K. Yamanouchia[2], A. Koretsky[3], H. Hetherington[4], C. Guha[2]

[1]Medicine, Albert Einstein College of Medicine, Bronx, New York, United States, [2]Radiation Oncology, Albert Einstein College of Medicine, Bronx, New York, United States, [3]Laboratory of Functional and Molecular Imaging, National Institute of Neurological Disorders and Stroke, Bethesda, Maryland, United States, [4]Radiology, Albert Einstein College of Medicine, Bronx, New York, United States

Introduction: Hepatocyte transplantation (HT) is being explored as a therapeutic alternative to organ transplantation for treatment of acute and chronic liver failure, inherited metabolic disorders, and liver cancers. Recently, we described a preparative regimen of hepatic irradiation (HIR) that enables transplanted hepatocytes to preferentially proliferate in irradiated host livers in response to hepatic mitogenic stimuli (1). However, engraftment, survival, proliferation and immune rejection of transplanted hepatocytes are difficult to monitor because of the invasive nature of liver biopsy procedures and spatial heterogeneity of repopulation of the liver by transplanted cells. These considerations underscore the need for developing robust non-invasive techniques that allow routine assessment of the survival, function and cell mass of the transplanted hepatocytes. Koretsky and others have described models in which ectopic expression of creatine kinase (CK) in liver enables ^{31}P MRS measurements of phosphocreatine (PCr), a metabolite not normally produced in liver (2,3). We hypothesized that transplanted CK expressing donor hepatocytes would enable ^{31}P MRSI to be used to monitor repopulation of the host liver after HT.

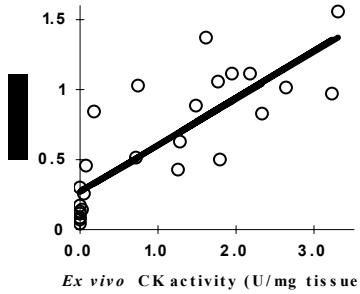
Methods: After exposing the liver by laparotomy and shielding the caudate lobe and other abdominal organs, BDF mice received partial HIR (50Gy) to anterior liver lobes. Six hours after HIR, mice received an injection of a recombinant adenoviral vector expressing human hepatocyte growth factor (Ad-HGF, 5-10 x 10^{10} particles). One day after HIR, intrasplenic HT was performed with 0.8-1x10^{6} primary hepatocytes, isolated from transgenic mice expressing brain isozyme CK (CK-BB) in liver (CK-Tg) under the transthyretin promoter (2). Control mice received Ad-HGF alone, or HIR + Ad-LacZ with HT. MR studies were performed 3, 4, and 8 months after HT. Each mouse received a diet supplemented with 10% creatine for 3-4 days prior to MRSI studies. MR data was acquired with a 9.4 T Varian INOVA MR system using a linear birdcage coil for ^{1}H MRI and a 2.5 cm surface coil for ^{31}P MRS. For ^{31}P MRSI, a 90° non-selective adiabatic hypersecant excitation and a 3D k-space sampling scheme on a 13×13×13 grid was used (FOV=32-48mm^3, TR=0.4-0.6s, 2 transients). After each MR study, mice were sacrificed and portions of the livers were assayed for CK enzyme activity and CK expression by immunoblot using standard techniques.

Figure 1.



Results and Discussion: Representative scout images and ROI positions for localized ^{31}P MRS spectra from ^{31}P MRSI studies of a) CK-Tg, b) control, and c) a mouse 4 months after HIR + Ad-HGF + HT with CK-Tg donor hepatocytes are shown in Figure 1. A PCr signal is clearly seen in liver ROIs for the CK-Tg mouse which is not present in the control liver, indicating expression of CK in the CK-Tg liver. In the mouse transplanted with CK-Tg hepatocytes, a PCr signal is also seen, but here it is due to expression of CK by the repopulating donor hepatocytes. To verify these results, *ex vivo* CK enzyme activity from median liver lobe homogenates were performed. Control mice that received HT after HIR+Ad-LacZ exhibited minimal CK activity and PCr signal, indicating engraftment of donor cells without repopulation due to the lack of mitotic stimulus provided by Ad-HGF treatment**.** Whereas, mice that received HIR+Ad-HGF+HT showed variable expression that depended on the total number and viability of the hepatocytes initially transplanted and the amount of time elapsed between transplantation and MR study. In order to compare detected PCr signal with CK expression, the PCr/ATP ratio was averaged over 2-3 ROIs (27µl voxels) within the median lode identified by the scout image. A regression analysis, pooling over all data, was then performed to evaluate the relationship between *in vivo* PCr/ATP (MRSI data) and *ex vivo* CK activity of the median lobe liver homogenate (Figure 2). Despite pooling across 25 different mice (normal BDF, CK-Tg and BDF that received HIR + Ad-HGF + HT, HIR + Ad-LacZ + HT, or Ad-HGF alone) a strong statistically significant relationship (Spearman R = +0.84, p<0.001) is seen.

Figure 2.



Conclusions: This is the first demonstration of the use of ^{31}P MRSI to evaluate the survival and proliferation of transplanted hepatocytes. The plot in Figure 2 shows a strong statistically significant correlation of ^{31}P MRSI PCr signal and liver CK expression. The data is normalized to ATP levels, which suggest that the energetic status of the mice receiving HT is also normal.

References:

1. Guha, et al., Am J Nephrol, 25: 161-170, 2005. **2**. Koretsky et al. Proc Natl Acad Sci U S A, *87:* 3112-3116, 1990. **3**. Auricchio et al. Proc Natl Acad Sci U S A, *98:* 5205-5210, 2001.