Title: Longitudinal Imaging and Functional Characterization of Liver Regeneration and Humanization in a Translational SRG Rat Model

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Hypothesis: Rodent models often fail to recapitulate human liver metabolism and regeneration, limiting their translational utility for liver cancer research. We aimed to establish human liver chimerism in immunodeficient SRG rats (Sprague Dawley Rag2/Il2rg–/–), which lack functional rodent T, B, and NK cells.

Methods: SRG rats were pretreated with retrorsine (30–45 mg/kg i.p. x 2 doses) to inhibit native rodent hepatocyte proliferation, followed by 70–80% partial hepatectomy (PH). Immediately after PH, rats received an intrasplenic injection of 3×10^6 viable human hepatocytes (HL rats, n=5), saline (n=3), or no injection (n=2). Liver regeneration was monitored using MRI volumetrics (axial and coronal T2-weighted imaging, ITK-SNAP segmentation) at weeks 2, 6, and 12, normalized to body weight. Plasma samples collected were analyzed for biochemical markers, bile acid profiles, and lipid concentrations using HDL and LDL/VLDL assay kits. Liver tissue was formalin-fixed for histological analyses or snap-frozen for proteomics.

Results: MRI demonstrated significantly greater liver regeneration post-PH in HL rats compared to controls at weeks 2 (3.34 ± 0.35% vs 2.81 ± 0.13%, p = 0.0034) and 6 (3.11 ± 0.23% vs 2.62 ± 0.13%, p = 0.0439). Liver weight/body weight ratio at necropsy (12 weeks post-PH) corroborated MRI findings (4.49 ± 0.36% vs 4.10 ± 0.21%, p = 0.0185). Serum ALT, albumin, and bilirubin levels were comparable, indicating preserved liver function. Bile acid profiling at weeks 6 and 12 demonstrated significant shifts toward human-specific metabolic patterns in HL rats, including increased glycine-conjugated bile acids (Week 12: GCA 10750 nM vs 1075 nM, GCDCA 1380 nM vs 138 nM), decreased taurine-conjugated bile acids (Week 12: TCA 870.6 nM vs 7835 nM, TCDCA 29.75 nM vs 238 nM), and substantially reduced rodent-specific muricholic acids (Week 12: MCA 3515 nM vs 464574 nM). Lipid profiling revealed substantial differences in LDL/HDL cholesterol ratios, with the humanized rat (HL12) showing a higher LDL/HDL ratio (1.51) compared to the control rat (0.36), closely resembling the human LDL/HDL profile (1.45), indicating human-specific lipid metabolism. Additional ongoing analyses include immunohistochemistry for human-specific lipoprotein A and human proteomics to further validate human hepatocyte engraftment.

Conclusion: This preliminary study establishes a foundational step toward a highly translational humanized rat model capable of supporting human hepatocyte engraftment, robust liver regeneration, and human-specific metabolic function. These findings offer crucial evidence for this model's potential in precisely modeling human liver biology and evaluating novel therapeutic strategies in liver disease.