

## Durable Persistence of the Fetal Hepatocyte Phenotype After Liver Cell Transplantation

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**Introduction:** Chronic Liver Disease (CLD) is a leading cause of death in the US and is increasing in incidence for adult and pediatric populations. The only current clinical treatment is orthotopic liver transplantation, which is hindered by the availability of donor livers suitable for transplant. Hepatic cell transplantation offers a potential alternative by using isolated cells, thereby relaxing reliance on whole livers. While adult hepatocytes have shown a limited window of efficacy, fetal hepatocytes have demonstrated in animal models and human clinical trials to durably persist and proliferate and correct hepatic deficiencies. Due to ethical and practical limitations sourcing fetal human hepatocytes, we are striving to understand the mechanisms by which fetal cells repopulate an injured liver, with the goal of enabling this phenotype in adult hepatocytes or induced cells. Our study examines histone post-translational modification (hPTM) and gene expression profiles of post-transplantation colonies, as well as those of primary fetal and adult liver cells. **Methods:** We used the Dipeptidyl Peptidase IV rat model to transplant fetal hepatocytes into adult hosts. After 10 months, livers were flash-frozen and cryosectioned. Using an enzyme activity stain and laser capture microdissection, we isolated sections of fetal-derived colonies and surrounding adult host hepatocytes. Separately, primary adult and fetal rat hepatocytes were isolated or immunopurified using a hepatic lineage marker. Histones and RNA were extracted from these tissue and cell samples for hPTM abundance quantification and RNA-Seq. Data was analyzed using R. **Results:** We have identified 13 distinct marks on Histone H3 that have significant differences in relative abundance in fetal-derived colonies vs. host tissue, as well as in fetal vs. adult hepatocytes. Our RNA-Seq study clustered post-transplant samples by their type (colony or host) rather than by biological replicate and resulted in 1,046 differentially expressed genes (DEGs) between colony and host. Ontological analysis of the gene set expressed higher in host tissue showed enriched metabolic processes, while the genes expressed higher in colonies showed enriched ion transmembrane transport. **Conclusions:** In both hPTM and mRNA expression, a distinct profile common to pre-transplant fetal cells is retained for at least 10 months following transplantation into an adult liver microenvironment. The 13 significantly different histone marks were all on Histone H3, which has known associations with gene transcription. The 186 DEGs in the host tissue population correspond to the incomplete metabolic maturity of the transplanted cells, while the 860 DEGs in the colonies reflect activities associated with their continued growth and proliferation activity. Our future work will explore the link between specific differentially expressed genes and the differentially abundant histone marks that may be regulating them.