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Concurrent Session 3

A method for lifelong genetic manipulation of regenerating hepatocytes in mouse

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Rapid and stable genetic engineering of the regenerating liver would allow systematic, *in vivo* testing of contributions by many genes to regenerating tissue. Following fumaryl acetoacetate hydrolase (Fah) gene transfer to hepatocytes, selective repopulation of the liver occurs in Fah-deficient mice. This genetic correction is readily mediated with transposons. Using this approach, we show that genes with biological utility can be linked to a selectable FAH transposon cassette. First, net conversion of Fah–/– liver tissue to transgenic tissue, and its outgrowth, was monitored by bioluminescence *in vivo* from a luciferase gene linked to the FAH gene. Second, co-expressed shRNAs stably reduced target gene expression, indicating the potential for loss-of-function assays. Third, a mutant, gain-of-function allele of human a1-antitrypsin (hAAT) was linked to Fah and resulted in protein inclusions within hepatocytes, which are the histopathological hallmark of hAAT deficiency disorder. Finally, oncogenes linked to Fah resulted in fulminant hepatocellular carcinoma. Conclusion: co-expression with FAH is an effective technique for expression of transgenes in regenerating adult hepatocytes with applicability to genetic studies of the liver stem cell repopulation.

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