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Evaluation of transcription factor activity using in vitro and in vivo models of chronic cholestatic liver disease

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ABSTRACT

Biliary obstruction results in cholestasis characterised by progressive fibrosis, cholangiocyte hyperplasia and cirrhosis ultimately leading to liver failure. Underlying molecular mechanisms remain unclear but likely involve deregulation of signalling pathways within bipotent progenitor cells. Transduction of cell lines with vectors containing serial transcription factor (TF) binding sequences upstream of a minimal promoter driving luciferase expression have been widely used to study TF activity *in vitro*. The presented data expands upon this technology both *in vitro* and *in vivo* to enable quantification of bioluminescent output in living cell cultures and animals. A library of lentiviral vectors expressing either firefly luciferase or secreted NanoLuc luciferase was generated. Validation was performed by agonist-mediated activation and subsequent luciferase readout. Human hepatocellular HepaRG cells can be cultured as bipotent progenitors capable of differentiating into cholangiocytes or hepatocytes, and are a valuable tool for understanding cholangiocyte hyperplasia. HepaRGs transduced with WNT, Notch, or alpha-1 antitrypsin (α1AT) NanoLuc reporters were temporally assayed from culture media during differentiation. Two alternative differentiation procedures were performed based on previously published protocols predicted to enrich for either hepatocytes or cholangiocytes. The effect of Notch signalling on differentiation potential was determined through Notch signalling modulation. Data indicate that constitutive expression of JAGGED-1 results in a predisposition toward the cholangiocyte lineage. Conversely, the percentage of hepatocyte-like cells increased with the suppression of Notch signalling by NUMB. This enrichment is synergistic when used in conjunction with the 3-component differentiation protocol. For *in vivo* experiments, various high-titer VSV-G pseudotyped lentiviruses containing the reporters were administered by intravascular injection to P0 neonatal mice. This resulted in liver-restricted transduction and lifelong tolerance of the transgene. After establishing a bioimaging baseline, adult mice were subjected to partial bile duct ligation to induce a cholestatic phenotype, followed by serial bioimaging. This permitted the evaluation and comparison of temporal activities of a number of candidate signalling pathways involved in the differentiation of hepatic progenitor cells *in vitro* and the response to cholestatic injury *in vivo*. These methods of longitudinal assessment are an improvement on current methodologies and can be used as tools to obtain mechanistic insight or as drug-screening platforms.