Int J Clin Exp Pathol 2017;10(9):9837-9845
www.ijcep.com /ISSN:1936-2625/IJCEP0056933

Original Article

Hedgehog signaling pathway regulates liver regeneration in the Fah$^{-/-}$ knockout mice model xenografted by human hepatocytes

Xiaoqing Xu[1], Xiaohu Jiao[2], Yuhong Jiao[1], Min Wang[1], Chenwei Jiao[3]

[1]Department of Radiation Oncology, Shandong Cancer Hospital Affiliated to Shandong University, Shandong Academy of Medical Sciences, Jinan, China; [2]Department of Surgery, Baoji Hospital Affiliated to Xi'an Medical University, Baoji, China; [3]Department of Pediatric Surgery, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, China

Received May 7, 2017; Accepted August 16, 2017; Epub September 1, 2017; Published September 15, 2017

Abstract: Background: The aim of this study is to evaluates the hypothesis that Hh pathway activation occurs in the Fah$^{-/-}$ Nod/Scid mice model and plays a role in regulating liver regeneration after functional human hepatocytes xenograft. Methods: *Fah$^{-/-}$ Nod/Scid* mice were established in Shandong Cancer Hospital affiliated to Shandong University. *Xeno-regeneration of Fah$^{-/-}$ Nod/Scid mice livers was then* transplanted by *human hepatocytes*. Hh signaling pathway associated factors were detected by RT-PCR and western blot. All statistical calculations were carried out using the SPSS.16.0 software. Results: *Fah$^{-/-}$* mice were healthy and reproduced normally while treated with NTBC. NTBC-OFF *Fah$^{-/-}$* mice with HHT gradually gained weight by six weeks after human hepatocyte transplantation. NTBC-OFF *Fah$^{-/-}$* mice causes a progressive increase in ALT, AST, GGT and AP, which were used to monitor hepatocyte inflammation. However, liver function in NTBC-OFF Fah *Fah$^{-/-}$* mice with HHT returned to normal. Hedgehog pathway activation and Hh-target genes were enhanced follows human hepatocytes transplantation which indicated liver regeneration associated closely with Hh signaling pathway. Moreover, Hh signaling was inhibited by cyclopamine after HHT. Conclusions: The current study provides novel evidence that Hedgehog signaling pathway regulation is required for optimal regeneration of NTBC *OFF Fah$^{-/-}$* mice with HHT.

Keywords: Hedgehog signaling, NTBC-OFF Fah$^{-/-}$ mice, human hepatocytes xenografted

Introduction

Fumarylacetoacetate hydrolase gene knockout mice (*Fah$^{-/-}$* mice) have been established as a model for hereditary tyrosinemia type I (HT1) disease [1], which is caused by a deficiency of fumarylacetoacetate hydrolase (FAH), the last enzyme of the tyrosine catabolic pathway [2, 3]. Meanwhile, since healthy livers have enormous regenerative capacity, many studies took insight into applying *Fah$^{-/-}$* mice model in studying liver damage and regeneration [4, 5].

Lack of FAH produces an accumulation of the toxic upstream metabolites fumarylacetoacetate (FAA), maleylacetoacetate (MAA), and succinylacetone (SA). FAA is believed to be responsible for the progressive injury to hepatocytes as it causes chromosomal instability [6, 7], cell death in animal and cultured cell models of the disease [8, 9]. NTBC (2-(2-nitro-4-trifluoromethyl benzoyl)-1,3-cyclohexanedione), a therapeutic inhibitor of the upstream enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD), prevents acute liver failure, a major cause of early death in HT1 patients.

Functional human hepatocytes xenografted into the liver of mice can be used as a model system to study pharmacokinetics, liver damage by hepatitis viruses, and the efficacy of hepatitis vaccines. Recently, robust liver regeneration from human hepatocytes was seen in *Fah$^{-/-}$Rag2$^{-/-}$Il2rg$^{-/-}$* mice [10-12]. However, maintenance of *Fah$^{-/-}$Rag2$^{-/-}$Il2rg$^{-/-}$* mice during colony breeding, growing and cell transplantation is difficult, with high mortality rates seen in previous experiments. Since *Fah$^{-/-}$* Non-obese diabetic-SCID (Nod/Scid) mice model was firstly established by Azuma *et al, [10] Fah$^{-/-}$ Nod/Scid*

mice are gradually becoming an ideal humanized liver model with many applications [13].

Hedgehog (Hh) is a signaling pathway that regulates critical cell fate decisions, including proliferation, apoptosis, migration and differentiation. The pathway plays vital roles in tissue morphogenesis during fetal development. It also modulates wound healing responses in a number of adult tissues, including the liver [14, 15]. Hh signaling is initiated by a family of ligands (Sonic hedgehog-Shh, Indian hedgehog -Ihh, and Desert hedgehog-Dhh) which interact with a cell surface receptor (Patched-PTC) that is expressed on Hh responsive target cells. This interaction derepresses activity of another molecule, Smoothened (Smo), and permits the propagation of intracellular signals that culminate in the nuclear localization of Glioblastoma (Gli) family transcription factors (Gli1, Gli2, Gli3) that regulate the expression of Gli-target genes.

The present study evaluates the hypothesis that Hh pathway activation occurs in the *Fah$^{-/-}$ Nod/Scid* mice model and plays a role in regulating liver regeneration after functional human hepatocytes xenograft. Our findings demonstrate the mechanisms of Hh pathway activation after human hepatocytes xenograft, and characterize the effects of Hh-pathway inhibition on the regenerative process. The results support our hypothesis and identify Hedgehog as a major regulator of liver regeneration in the fah$^{-/-}$ knockout mice model xenografted by human hepatocytes. This, in turn, suggests that common mechanisms regulate liver growth during organogenesis and when reconstruction of adult livers is necessitated by injury.

Materials and methods

Establishment of Fah$^{-/-}$ Nod/scid mice and experimental grouping

Fah$^{+/+}$ mice, Fah$^{-/-}$ mice and Nod/Scid mice were obtained from Shandong Cancer Hospital affiliated to Shandong University. All types of mice were raised in an individual ventilated cage system in the specific pathogen free grade animal room in the animal center of Shandong University. Fah$^{-/-}$ mice was crossed with Nod/Scid mice to establish the Fah$^{-/-}$ Nod/Scid mouse strain [16]. PCR-based genotyping for Fah and SCID was used to determine the genotypes of the offspring. The method for Fah$^{-/-}$ Nod/Scid mice establishment was according to the previously published reports [1].

Different types of mice divided into four groups:1) control untreated fah$^{+/+}$ mice (n = 10); 2) NTBC-treated Fah$^{-/-}$ mice (n = 10) were used to underline cellular features exclusively related to NTBC withdrawal; 3) Fah$^{-/-}$ mice by NTBC discontinuation from 1 to 5 weeks (10 mice per period); 4) Fah$^{-/-}$ mice without NTBC and with human hepatocytes transplantation from 1 to 12 weeks (10 mice per period). All mice were allowed access to regular rodent food (0.82% tyrosine, 0.51% phenylalanine, Purina 5075-U.S, Charles River, Canada) and drinking water, supplemented with 7.5 mg/l NTBC, pH 7 for the Fah$^{-/-}$ strain. Mice were weighed three times a week, periodically examined for signs of clinical illness, and treated according to the guidelines of the Canadian Council on Animal Care (CCAC).

Xeno-regeneration of Fah$^{-/-}$ Nod/Scid mice livers by human hepatocytes

Human liver tissue was provided by Shandong Cancer Hospital affiliated to Shandong University. The method used to separate human hepatocytes has been described previously [17]. 40 Fah$^{-/-}$ Nod/Scid mice were chosen for human hepatocytes transplantation. Seven days before transplantation, Human hepatocytes (1×106) were injected into the spleens of 14 Fah$^{-/-}$ Nod/Scid mice. Six Fah$^{-/-}$ Nod/Scid mice were injected normal saline as the control group. Gradual withdrawal of NTBC one week before transplantation caused liver injury in Fah$^{-/-}$ Nod/Scid mice and induced the engraftment of transplanted human hepatocytes. At the same time, mice were given FK506 to promote the proliferation of human hepatocytes. The level of NTBC in the drinking water was reduced to 50% for 3 d, and further reduced to 25% for 3 d. NTBC was discontinued 2 d before transplantation. This treatment induced liver injury ahead of schedule, and promoted the engraftment and proliferation of the transplanted cells. FK506 (Astellas Ireland Co.; 7.5 μg ml^{-1}) was dissolved in the drinking water and administered to adult mice to achieve a dose of 1 μg g^{-1} body weight per day.

Biochemical analysis of serum in humanized liver mice

Progression of hepatic damages was evaluated by changes of alkaline phosphatase, c-glutamyl-transferase and alanine transaminase

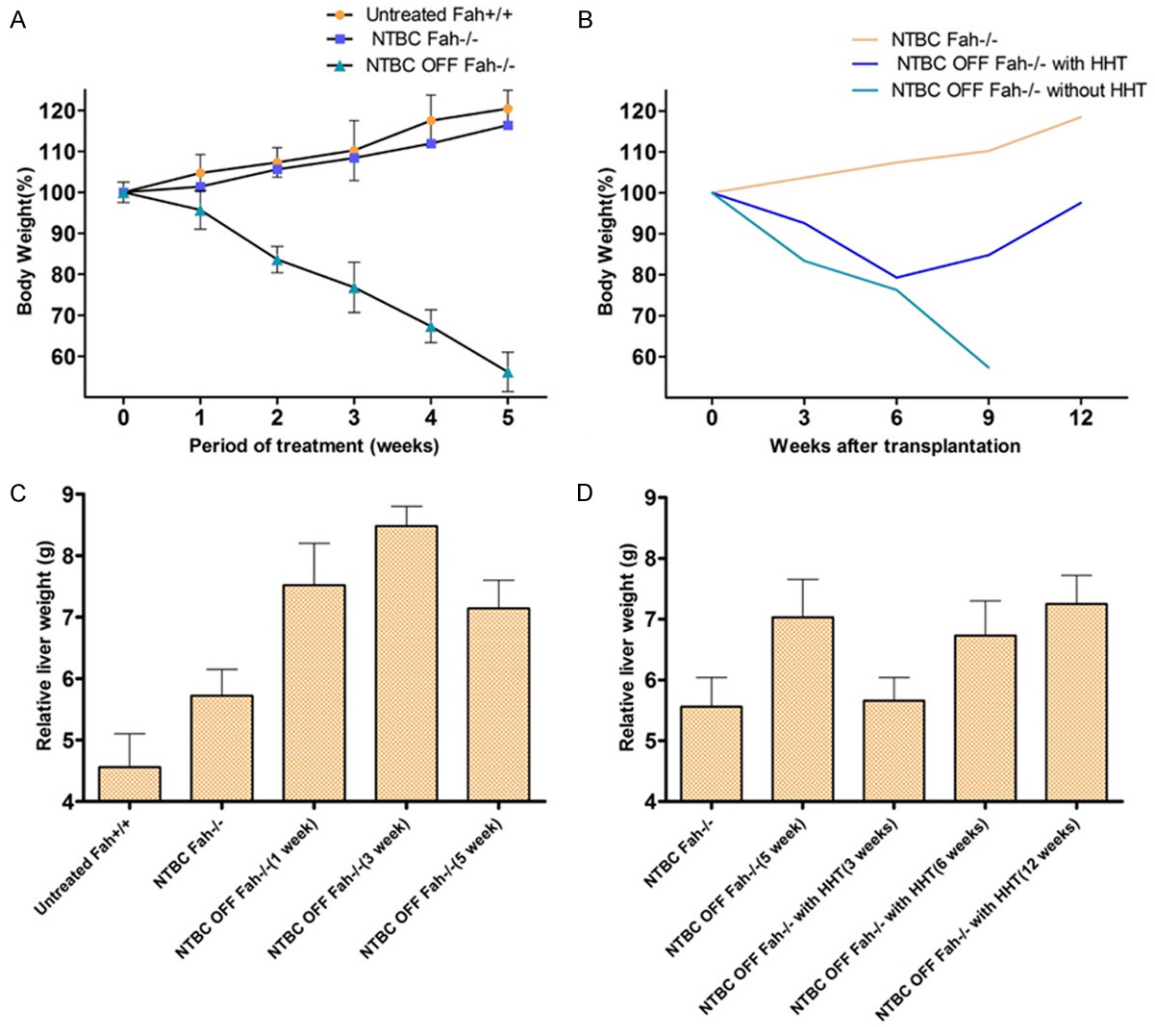

Figure 1. Body and liber weight curves (%) for NTBC-OFF *Fah*$^{-/-}$ mice with or without HHT. Mice were weighed to evaluate general physiological conditions.

(liver), and urea and creatinine (kidneys) in samples of mice withdrawn from NTBC for different periods of time (1-5 weeks). Levels of bilirubin and glucose were also measured.

Real-time quantitative PCR

We typically extracted 2 μg to 9 μg of total RNA, and OD260/280 ratios typically ranged from 1.8 to 2.0, indicating high RNA purity. 10 ng of total RNA was used for each miRNA quantification. miRNA detection was performed run on the Eppendorf Mastercycler EP Gradient S (Eppendorf, Germany) using commercial assays (TaqMan microRNA assays; Applied Biosystems, Foster City, CA, USA) for miRNAs. Relative quantification was calculated using $2^{-\Delta\Delta Ct}$, where Ct is cycle threshold. Normalization was performed with universal small nuclear RNA U6 (RNU6B). Each sample was examined in triplicate, and the mean values were calculated.

Western blot

Protein extracts were prepared by whole liver tissue or primary hepatocytes in RIPA buffer (R0278; Sigma) and quantified by Pierce BCA kit. After quantification, equal amounts of protein were separated by SDS-PAGE and Western blot analysis was performed. The following antibodies were used: Ptc, Smo, Gli1 and Gli2 (Cell Signaling Technology Inc., Beverly, MA), we also used β-actin as a loading control.

Inhibition of the Hh-pathway

To determine if inhibiting the Hh-pathway altered liver regeneration of *Fah*$^{-/-}$ *Nod/Scid* mice

Table 1. Biochemical analysis in blood serum

FAH	No. of patients	Bilirubin (μmol/L)	ALT (U/L)	AST (U/L)	GGT (U/L)	AP (U/L)
Untreated Fah$^{+/+}$	10	3.5 ± 0.6	23.5 ± 12.1	43.1 ± 23.2	3.5 ± 4.2	57.1 ± 15.4
NTBC Fah$^{-/-}$	10	4.3 ± 1.1	28.7 ± 17.7	55.8 ± 12.7	4.7 ± 2.6	46.7 ± 17.6
NTBC OFF Fah$^{-/-}$ (1 week)	10	15.3 ± 2.6	56.6 ± 13.4	83.5 ± 23.9	14.3 ± 7.8	176.6 ± 27.8
NTBC OFF Fah$^{-/-}$ (3 week)	10	32.7 ± 4.4	167.3 ± 34.2	215.4 ± 15.5	45.7 ± 6.4	266.9 ± 27.3
NTBC OFF Fah$^{-/-}$ (5 week)	10	76.5 ± 3.8	385.2 ± 21.4	476.7 ± 34.8	85.2 ± 7.9	512.5 ± 43.7
NTBC OFF Fah$^{-/-}$ with HHT (3 weeks)	10	16.7 ± 2.4	107.3 ± 15.6	157.6 ± 27.1	45.2 ± 7.1	175.3 ± 25.5
NTBC OFF Fah$^{-/-}$ with HHT (6 weeks)	10	7.3 ± 2.1	76.8 ± 20.7	85.3 ± 20.5	26.1 ± 8.1	84.5 ± 31.6
NTBC OFF Fah$^{-/-}$ with HHT (12 weeks)	10	4.5 ± 0.8	43.5 ± 19.8	61.5 ± 14.7	10.5 ± 6.3	60.7 ± 21.8

ALT, alanine transaminase; AST, aspartate aminotransferase; GGT, c-glutamyl-transferase; AP, alkaline phosphatase.

xenografted with human hepatocytes, human hepatocytes transplantation was performed in an additional 30 *Fah*$^{-/-}$ *Nod/Scid* mice (10-13-week old males) that were injected i.p. with vehicle (olive oil) or cyclopamine (15 mg/kg/day) 24 h before human hepatocytes transplantation and daily thereafter. Liver remnants and blood were harvested for subsequent analysis. In addition to evaluating Hhsignaling, potential toxic actions of cyclopamine were assessed by examining liver histology and levels of alanine transaminase; aspartate aminotransferase; c-glutamyl-transferase; bilirubin and alkaline phosphatase.

Statistical analysis

All statistical calculations were carried out using the SPSS.16.0 software. The χ^2 test or Fisher's exact test were used to compare qualitative variables, while continuous variables were compared using unpaired, two-tailed Student's *t*-test or Mann-Whitney test for variables with an abnormal distribution. Statistical significance was determined using one-way ANOVA. Statistical significance was determined using Significance was accepted at the 5% level.

Results

Pathophysiological changes in different types of mice

Fah$^{-/-}$ mice were healthy and reproduced normally while treated with NTBC. Once NTBC was withdrawn, *Fah*$^{-/-}$ mice lost weight gradually and died of subacute liver failure after 3-6 weeks. The body weight and liver weight could demonstrate the liver injury and the pathophysiological effect of NTBC. The *Fah*$^{-/-}$ mice withdrawn from NTBC (NTBC-OFF) are subjected to a severe loss of total body mass reaching from 100 ± 2.5% to 56.8 ± 4.8% during 5 weeks post-withdrawal of NTBC. In contrast, during the same period, *Fah*$^{+/+}$ and NTBC-treated *Fah*$^{-/-}$ mice display weight gains of 20.4 ± 4.5% and 16.4 ± 5.2%, respectively (**Figure 1A**). In the present study, we also found that *Fah*$^{-/-}$ mice withdrawal NTBC and transplanted with human hepatocytes (NTBC-OFF *Fah*$^{-/-}$ mice with HHT) gradually gained weight by six weeks after human hepatocyte transplantation, indicating recovery of liver function and high levels of liver regeneration in these mice (**Figure 1B**). Similarly, the present study showed that liver weight of NTBC-OFF *Fah*$^{-/-}$ mice decreased with the lapse of time, while, the liver weight of NTBC-OFF *Fah*$^{-/-}$ mice with HHT increased during the same period (**Figure 1C**, **1D**).

Changing trend of liver function of NTBC-OFF Fah$^{-/-}$ *mice with HHT*

Biochemical measurements of several damage markers are reported in **Table 1**. Discontinuation of NTBC in *Fah*$^{-/-}$ mice causes a progressive increase in ALT, AST, GGT and AP, which were used to monitor hepatocyte inflammation. However, liver function in NTBC-OFF *Fah*$^{-/-}$ mice with HHT returned to normal which indicated that percentage of functional human hepatocytes in the chimeric liver had exceeded 20%.

Hedgehog pathway activation follows human hepatocytes transplantation

Hepatic expression of mRNAs that encode Hh ligands (Indian Hh, Ihh, and Sonic Hh, Shh), the receptors that repress (Patched, Ptc) or promote (Smoothened, Smo) Hh signaling, and

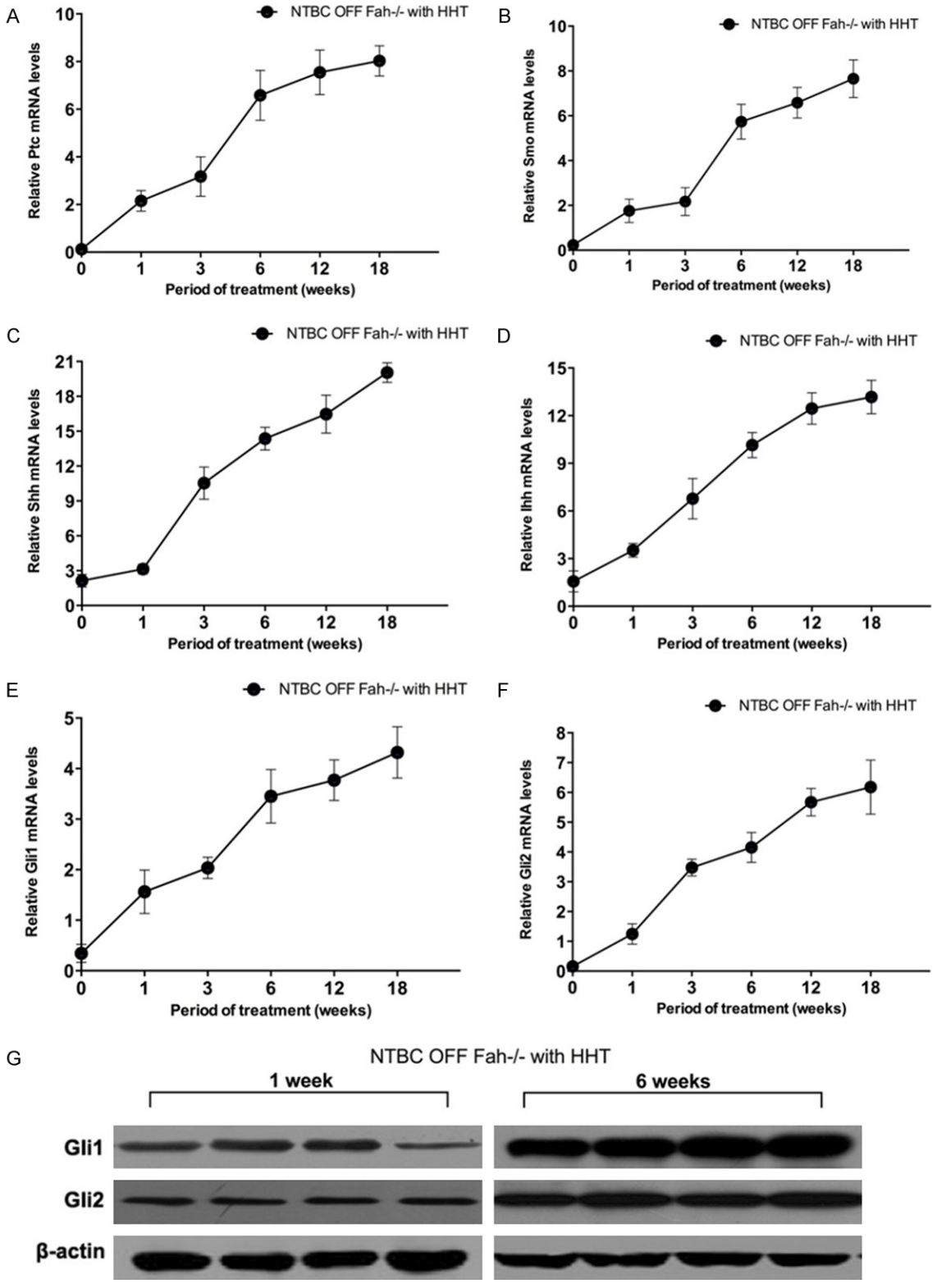

Figure 2. Activation of the Hedgehog pathway after liver xeno-regeneration in *Fah*$^{-/-}$ *Nod/Scid* mice by human hepatocytes.

Hh-inducible transcription factors (Glioblastoma (Gli)1 and Gli2) were evaluated at several distinct time points in NTBC-OFF *Fah*$^{-/-}$ mice with HHT. For each gene, results were normalized to

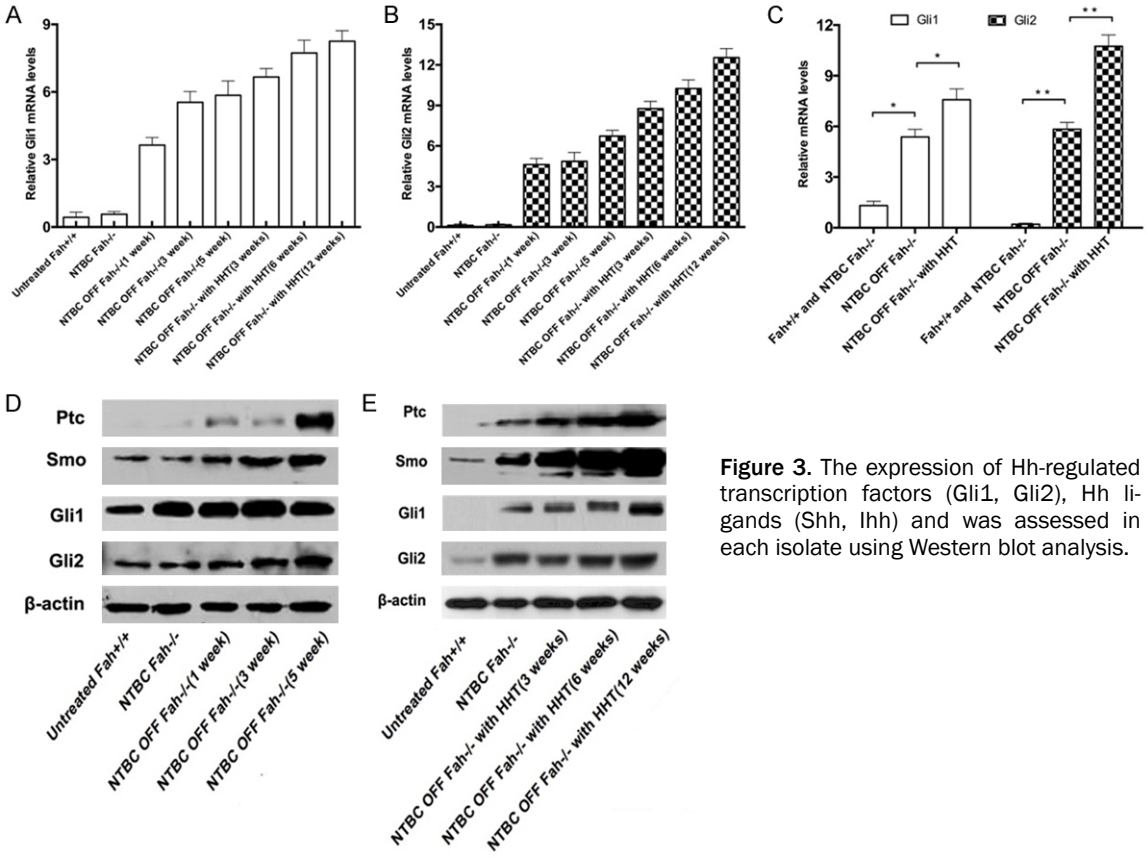

Figure 3. The expression of Hh-regulated transcription factors (Gli1, Gli2), Hh ligands (Shh, Ihh) and was assessed in each isolate using Western blot analysis.

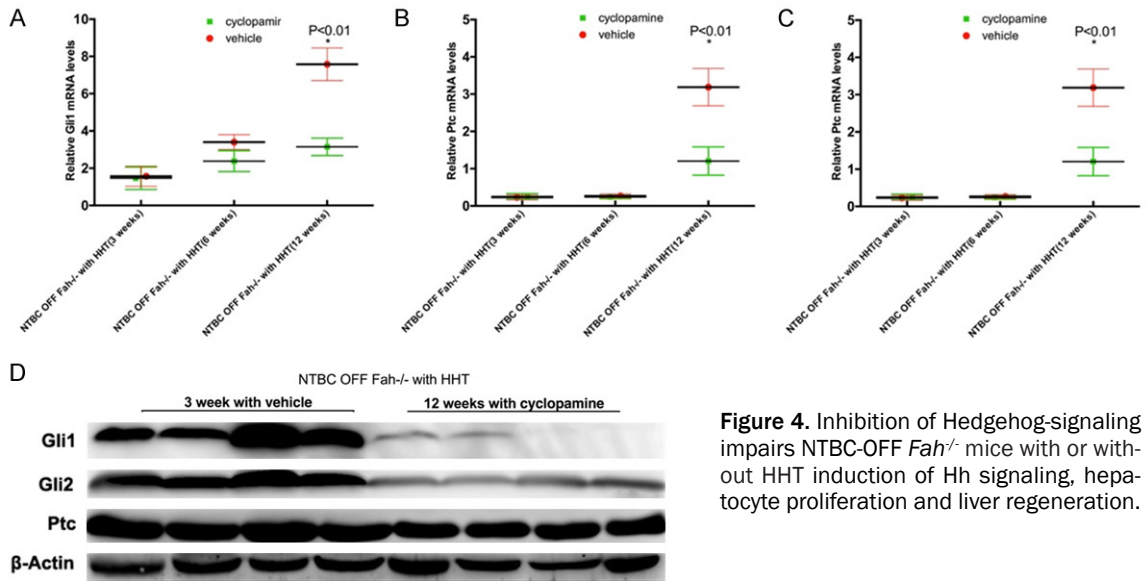

Figure 4. Inhibition of Hedgehog-signaling impairs NTBC-OFF *Fah*$^{-/-}$ mice with or without HHT induction of Hh signaling, hepatocyte proliferation and liver regeneration.

expression in the liver at the time of NTBC reduced and discontinued for 1 week and graphed as a function of time to demonstrate how gene expression varied during the pre-regeneration period, period of maximal liver cell regeneration, and post regeneration period (**Figure 2**). HHT of NTBC-OFF *Fah*$^{-/-}$ mice was accompanied by increased expression of Hh ligands. mRNA levels of Ihh began to increase during the pre-regeneration period, remained at their highest values during the regeneration period, no declining trend had been showed in

the post-regeneration period. The relative abundance of Ptc and Smo mRNAs changed after HHT, such that expression of Smo (the signaling competent Hh co-receptor) was increasing consistently compared with that of Ptc (the inhibitory Hh receptor) throughout the hepatocyte proliferation periods. Together with the reciprocal changes in mRNA expression of Hh ligand antagonists and Hh ligands, the predominance of Smo relative to Ptc suggested that Hh signaling would increase liver regeneration. Changes in expression of Gli1 and Gli2 support this concept.

Hepatocytes cells express Hh-target genes after HHT

In the groups of *untreated Fah$^{+/+}$ and* NTBC-treated *Fah$^{-/-}$* mice, Hh-target genes, such as Gli1 and Gli2, expressed less in mature human hepatocytes than those in the groups of NTBC OFF Fah$^{-/-}$ mice and NTBC OFF Fah$^{-/-}$ mice with HHT (**Figure 3A-C**). After further comparison, NTBC *OFF Fah$^{-/-}$* mice with HHT showed significant increasing Gli1 and Gli2 expression which was implicated with Hh signaling pathway activation and liver regeneration. Meanwhile, expression of Ptc and Smo showed consistent tendency compared among different groups (**Figure 3D**, **3E**).

Hh signaling was inhibited by cyclopamine after HHT

To determine how Hh-pathway activation impacts regenerative responses HHT, mice were treated with cyclopamine, a specific Smo antagonist that abrogates Hh signal transduction [18] or vehicle (olive oil) at the beginning of HHT (3 weeks) and post-HHT (12 weeks). As expected, cyclopamine attenuated induction of Gli1 and Gli2 mRNAs (**Figure 4A**, **4B**) and proteins (**Figure 4D**), and inhibited mRNA/protein expression of Ptc (**Figure 4C**, **4D**). However, cyclopamine did not prevent induction of Hh ligands (data not shown).

Discussion

Compared to other vital organs, the adult liver has tremendous regenerative capacity. Yet, hepatic regenerative responses are not always successful because liver injury sometimes results in progressive damage that leads to cirrhosis or cancer [19]. human hepatocytes engrafted into *Fah$^{-/-}$ Nod/Scid* mice were applied as a possible model for performing research on liver regeneration of liver injury [13, 20]. In the present study, we combined the advantages of liver regeneration seen in *Fah$^{-/-}$* mice with the ease of xenotransplantation seen in *Nod/Scid* mice to produce *Fah$^{-/-}$ Nod/Scid* mice by a gradual process of cross-breeding. After establishment of NTBC-OFF *Fah$^{-/-}$* mice with HHT, we demonstrated that the body and liver weight of NTBC-OFF *Fah$^{-/-}$* mice decreased with the lapse of time, while, the liver weight of NTBC-OFF *Fah$^{-/-}$* mice with HHT increased during the same period, which reflected the extent of liver regeneration from one side. Besides, compared to mice on NTBC, at week-5 post-withdrawal, ALT raised to 385.2 ± 21.4 U/L (10-fold), AST to 476.7 ± 34.8 U/L (12.3-fold) and GGT to 85.2 ± 7.9 U/L (18.5-fold), suggesting hepatocyte damage and/or necrosis and extensive liver dysfunction. Bilirubin and alkaline phosphatase (AP) also show noticeable increases mainly after 2 weeks of NTBC withdrawal, confirming hepatocellular dysfunction and disruption of hepatobiliary architecture with local impairment of bile flow [21, 22]. However, after transplanted with human hepatocytes, the liver function recovered to normal, which indicated that liver has a strong compensatory ability and increasing liver regeneration level. Moreover, NTBC *OFF Fah$^{-/-}$* mice with HHT showed significant increasing Gli1 and Gli2 expression which was implicated with Hh signaling pathway activation and liver regeneration.

Emerging data indicate that hedgehog signaling mediates both adaptive and maladaptive responses to liver injury, depending upon the balance between its actions as a regulator of progenitor cell growth and its ability to promote liver regeneration [23, 24]. The evidence that healthy adult livers general lacked of Hh pathway activity included: firstly, little production of Hh ligands is demonstrable in healthy adult liver cells [25, 26]. Secondly, liver sinusoidal cells strongly express Hip, which interacts with soluble Hh ligands and prevents them from engaging Ptc [27, 28]. Thirdly, Hh pathway activity is progressively silenced during the process of liver epithelial cell maturation, such that expression of Ptc1 is exponentially lower in healthy mature hepatocytes than in bipotent hepatic progenitors [29]. Consistent with these data, Hh pathway activation in hepatocytes increased significantly after 70% partial hepatectomy (PH) which provides a tremendous

stimulus for liver regeneration [30]. Several key growth regulators for this process have been identified, including hepatocyte mitogens, cytokines, pathogen-associated molecular pattern (PAMP) receptors, and intracellular factors involved in inflammatory and metabolic stress [30].

This study demonstrated that Hh pathway activation was critical for liver regeneration to NTBC *OFF Fah*$^{-/-}$ mice with HHT. Hepatic expression of mRNAs that encode Ihh, and Shh, Ptc or Smo Hh signaling, Gli1 and Gli2 were evaluated at several distinct time points in NTBC-OFF *Fah*$^{-/-}$ mice with HHT. For each gene, results were normalized to expression in the liver at the time of NTBC reduced and discontinued for 1 week. The enhancement of these genes illustrated the activation of Hh pathway in NTBC *OFF Fah*$^{-/-}$ mice with HHT. After inhibited by cyclopamine, Hh-target genes Gli1 and Gli2 mRNAs and proteins decreased significantly in NTBC *OFF Fah*$^{-/-}$ mice with HHT.

However, many different types of cells that reside in healthy livers collaborate with one another to orchestrate effective regeneration. In order to optimize regeneration in injured livers, more knowledge is needed about mechanisms that control the growth of hepatocytes and other liver cell types in adults. More research is needed to characterize the types of cells, cell-type specific responses, and particular aspects of liver reconstruction that are most dependent upon Hh signaling in order to judge the potential merits of manipulating Hh pathway activity to improve adult liver repair.

In conclusion, the current study provides novel evidence that Hedgehog signaling pathway regulation is required for optimal regeneration of NTBC *OFF Fah*$^{-/-}$ mice with HHT. This discovery complements growing evidence that Hh signaling guides repair of chronically injured livers, and is exciting because it suggests that common mechanisms mediate liver injury. Such knowledge has important implications for patients with various types of acute and chronic liver damage.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Chenwei Jiao, Department of Pediatric Surgery, Shandong Provincial Hospital Affiliated to Shandong University, No. 324 Jingwu Road, Jinan 250021, Shangdong, China. E-mail: chentaosyu@126.com

References

[1] Grompe M, al-Dhalimy M, Finegold M, Ou CN, Burlingame T, Kennaway NG and Soriano P. Loss of fumarylacetoacetate hydrolase is responsible for the neonatal hepatic dysfunction phenotype of lethal albino mice. Genes Dev 1993; 7: 2298-2307.

[2] Tanguay RM, Valet JP, Lescault A, Duband JL, Laberge C, Lettre F and Plante M. Different molecular basis for fumarylacetoacetate hydrolase deficiency in the two clinical forms of hereditary tyrosinemia (type I). Am J Hum Genet 1990; 47: 308-316.

[3] Phaneuf D, Lambert M, Laframboise R, Mitchell G, Lettre F and Tanguay RM. Type 1 hereditary tyrosinemia. Evidence for molecular heterogeneity and identification of a causal mutation in a French Canadian patient. J Clin Invest 1992; 90: 1185-1192.

[4] Li L, Zeng Z, Qi Z, Wang X, Gao X, Wei H, Sun R and Tian Z. Natural killer cells-produced IFN-gamma improves bone marrow-derived hepatocytes regeneration in murine liver failure model. Sci Rep 2015; 5: 13687.

[5] Qi Z, Wang X, Wei H, Sun R and Tian Z. Infiltrating neutrophils aggravate metabolic liver failure in fah-deficient mice. Liver Int 2015; 35: 774-785.

[6] Jorquera R and Tanguay RM. Cyclin B-dependent kinase and caspase-1 activation precedes mitochondrial dysfunction in fumarylacetoacetate-induced apoptosis. FASEB J 1999; 13: 2284-2298.

[7] Jorquera R and Tanguay RM. Fumarylacetoacetate, the metabolite accumulating in hereditary tyrosinemia, activates the ERK pathway and induces mitotic abnormalities and genomic instability. Hum Mol Genet 2001; 10: 1741-1752.

[8] Endo F, Kubo S, Awata H, Kiwaki K, Katoh H, Kanegae Y, Saito I, Miyazaki J, Yamamoto T, Jakobs C, Hattori S and Matsuda I. Complete rescue of lethal albino c14CoS mice by null mutation of 4-hydroxyphenylpyruvate dioxygenase and induction of apoptosis of hepatocytes in these mice by in vivo retrieval of the tyrosine catabolic pathway. J Biol Chem 1997; 272: 24426-24432.

[9] Kubo S, Sun M, Miyahara M, Umeyama K, Urakami K, Yamamoto T, Jakobs C, Matsuda I and Endo F. Hepatocyte injury in tyrosinemia type 1 is induced by fumarylacetoacetate and is inhibited by caspase inhibitors. Proc Natl Acad Sci U S A 1998; 95: 9552-9557.

[10] Azuma H, Paulk N, Ranade A, Dorrell C, Al-Dhalimy M, Ellis E, Strom S, Kay MA, Finegold M and Grompe M. Robust expansion of human hepatocytes in Fah-/-/Rag2-/-/Il2rg-/- mice. Nat Biotechnol 2007; 25: 903-910.

[11] Bissig KD, Le TT, Woods NB and Verma IM. Repopulation of adult and neonatal mice with human hepatocytes: a chimeric animal model. Proc Natl Acad Sci U S A 2007; 104: 20507-20511.

[12] He Z, Zhang H, Zhang X, Xie D, Chen Y, Wangensteen KJ, Ekker SC, Firpo M, Liu C, Xiang D, Zi X, Hui L, Yang G, Ding X, Hu Y and Wang X. Liver xeno-repopulation with human hepatocytes in Fah-/-Rag2-/- mice after pharmacological immunosuppression. Am J Pathol 2010; 177: 1311-1319.

[13] Su B, Liu C, Xiang D, Zhang H, Yuan S, Wang M, Chen F, Zhu H, He Z, Wang X and Hu Y. Xeno-repopulation of Fah-/-Nod/Scid mice livers by human hepatocytes. Sci China Life Sci 2011; 54: 227-234.

[14] Hooper JE and Scott MP. Communicating with hedgehogs. Nat Rev Mol Cell Biol 2005; 6: 306-317.

[15] Varjosalo M, Li SP and Taipale J. Divergence of hedgehog signal transduction mechanism between Drosophila and mammals. Dev Cell 2006; 10: 177-186.

[16] Grompe M, Lindstedt S, al-Dhalimy M, Kennaway NG, Papaconstantinou J, Torres-Ramos CA, Ou CN and Finegold M. Pharmacological correction of neonatal lethal hepatic dysfunction in a murine model of hereditary tyrosinaemia type I. Nat Genet 1995; 10: 453-460.

[17] Wang X, Montini E, Al-Dhalimy M, Lagasse E, Finegold M and Grompe M. Kinetics of liver repopulation after bone marrow transplantation. Am J Pathol 2002; 161: 565-574.

[18] Chen JK, Taipale J, Cooper MK and Beachy PA. Inhibition of Hedgehog signaling by direct binding of cyclopamine to Smoothened. Genes Dev 2002; 16: 2743-2748.

[19] Swiderska-Syn M, Xie G, Michelotti GA, Jewell ML, Premont RT, Syn WK and Diehl AM. Hedgehog regulates yes-associated protein 1 in regenerating mouse liver. Hepatology 2016; 64: 232-244.

[20] Naito A, Azuma S, Tanaka S, Miyazaki T, Takaki S, Takatsu K, Nakao K, Nakamura K, Katsuki M, Yamamoto T and Inoue J. Severe osteopetrosis, defective interleukin-1 signalling and lymph node organogenesis in TRAF6-deficient mice. Genes Cells 1999; 4: 353-362.

[21] Verslype C. Evaluation of abnormal liver-enzyme results in asymptomatic patients. Acta Clin Belg 2004; 59: 285-289.

[22] Green RM and Flamm S. AGA technical review on the evaluation of liver chemistry tests. Gastroenterology 2002; 123: 1367-1384.

[23] Sicklick JK, Li YX, Choi SS, Qi Y, Chen W, Bustamante M, Huang J, Zdanowicz M, Camp T, Torbenson MS, Rojkind M and Diehl AM. Role for hedgehog signaling in hepatic stellate cell activation and viability. Lab Invest 2005; 85: 1368-1380.

[24] Yang L, Wang Y, Mao H, Fleig S, Omenetti A, Brown KD, Sicklick JK, Li YX and Diehl AM. Sonic hedgehog is an autocrine viability factor for myofibroblastic hepatic stellate cells. J Hepatol 2008; 48: 98-106.

[25] Jung Y, McCall SJ, Li YX and Diehl AM. Bile ductules and stromal cells express hedgehog ligands and/or hedgehog target genes in primary biliary cirrhosis. Hepatology 2007; 45: 1091-1096.

[26] Omenetti A, Popov Y, Jung Y, Choi SS, Witek RP, Yang L, Brown KD, Schuppan D and Diehl AM. The hedgehog pathway regulates remodelling responses to biliary obstruction in rats. Gut 2008; 57: 1275-1282.

[27] Choi SS, Omenetti A, Witek RP, Moylan CA, Syn WK, Jung Y, Yang L, Sudan DL, Sicklick JK, Michelotti GA, Rojkind M and Diehl AM. Hedgehog pathway activation and epithelial-to-mesenchymal transitions during myofibroblastic transformation of rat hepatic cells in culture and cirrhosis. Am J Physiol Gastrointest Liver Physiol 2009; 297: G1093-1106.

[28] Choi SS, Witek RP, Yang L, Omenetti A, Syn WK, Moylan CA, Jung Y, Karaca GF, Teaberry VS, Pereira TA, Wang J, Ren XR and Diehl AM. Activation of Rac1 promotes hedgehog-mediated acquisition of the myofibroblastic phenotype in rat and human hepatic stellate cells. Hepatology 2010; 52: 278-290.

[29] Sicklick JK, Li YX, Melhem A, Schmelzer E, Zdanowicz M, Huang J, Caballero M, Fair JH, Ludlow JW, McClelland RE, Reid LM and Diehl AM. Hedgehog signaling maintains resident hepatic progenitors throughout life. Am J Physiol Gastrointest Liver Physiol 2006; 290: G859-870.

[30] Ochoa B, Syn WK, Delgado I, Karaca GF, Jung Y, Wang J, Zubiaga AM, Fresnedo O, Omenetti A, Zdanowicz M, Choi SS and Diehl AM. Hedgehog signaling is critical for normal liver regeneration after partial hepatectomy in mice. Hepatology 2010; 51: 1712-1723.