Prenatal Glucocorticoid Administration Accelerates the Maturation of Fetal Rat Hepatocyte

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Abstract

Prenatal glucocorticoid (GC) is clinically administered to pregnant women who are at risk of preterm birth for maturation of cardiopulmonary function. Preterm and low-birth-weight infants often experience liver dysfunction after birth because the liver is immature. However, the effects of prenatal GC administration on the liver remain unclear. We aimed to investigate the effects of prenatal GC administration on the maturation of liver hepatocytes in preterm rats.

Dexamethasone (DEX) was administered to pregnant Wistar rats on gestational days 17 and 19 before cesarean section. Real time-polymerase chain reaction (RT-PCR) was performed to determine the mRNA levels of albumin, HNF4 α , HGF, Thy-1, cyclin B, and CDK1 in the liver samples. Immunohistochemical staining and enzyme-linked immunosorbent assay were performed to examine protein production.

The hepatocytes enlarged because of growth and prenatal DEX administration. Albumin, HNF4 α , and HGF levels increased secondary to growth and prenatal DEX administration. The levels of the cell cycle markers cyclin B and CDK1 gradually decreased during growth and with DEX administration.

The results suggest that prenatal GC administration leads to hepatocyte maturation via expression of HNF4 α and HGF in premature fetuses.

Introduction

The prevalence rates of low-birth-weight and preterm infants are high among developed countries, especially in Japan. Therefore, research focusing on the fetal and childhood periods is advocated [1]. Preterm birth occurs before the physiological increase in endogenous glucocorticoid (GC) levels. GC action is important in the structural and functional maturation of various organs [2, 3].

Prenatal GC administration in mothers with infants at risk of delivery between 26 and 32 weeks of gestation is commonly used to accelerate pulmonary circulation and reduce the incidence of mortality [4]. We previously demonstrated that prenatal GC administration promotes growth and development of cardiac functions in premature infant rats [5-7]. The neonatal liver is generally immature and develops structure and function during the early postnatal period. However, preterm infants with immature liver function are at risk of hypoglycemia, hyperbilirubinemia, cholestasis, bleeding, and impaired drug metabolism [8]. Furthermore, low-birth-weight and preterm infants often develop physiological jaundice with immature fetal liver function [8-9].

The role of GC has been demonstrated in association with the differentiation of hepatocytes and the bile duct [10-11]. Dexamethasone (DEX) is administered for the differentiation of hepatic stem progenitor cells established from induced pluripotent stem cells into hepatocytes and their subsequent maturation [12].

In contrast, repeated administration of prenatal GC has been reported to decrease the liver weight of mice [13]. However, there is no explanation regarding the development of the fetal liver subsequent to prenatal GC administration.

Therefore, we predicted that prenatal GC administration would promote liver maturation during fetal development. Fetal hepatocytes are structurally immature and have many stem cells. Various factors have been associated with maturation in the fetal period [14].

The expression levels of various enzymes, such as tyrosine aminotransferase (TAT), glucose 6-phosphatase (G6Pase), and uridine diphosphate–glucuronosyltransferase (UGT) 1A, increase rapidly from the late embryonic period to birth [15–17].

In this study, we aimed to determine the process of differentiation and maturation of hepatocytes that accompanies growth in the fetal liver.

Material And Methods

1) Animal experiments

We determined the dose of GC based on previous reports [5–7]. Wistar rats were purchased from CLEA (Tokyo, Japan). DEX (Fujifilm Wako Pure Chemical, Osaka, Japan) dissolved in sesame oil (Kanto Chemical, Tokyo)—at doses of 1.0 and 2.0 mg/kg—was administered subcutaneously on days 17 and 19 of the gestation period to 8-week-old pregnant Wistar rats. In clinical practice, two doses of DEX have been administered every 24 h. Samatani et al. administered a total dose of 6 µM/kg in pregnant rats and described the optimal regimen of DEX for fetal lung maturation during the late gestation period using pharmacokinetic/pharmacodynamics simulation [18, 19]. We determined the dose of DEX by referring to these previous literatures. After mating, vaginal smears were taken every morning; the day of finding sperm was designated as day 1 of pregnancy. The pregnant rats gave birth to 10–14 neonates on day 22 of gestation. On days 19 and 21, fetal rats were delivered by cesarean section, and liver samples were extracted under inhaled isoflurane anesthesia. To evaluate the change the factor with growth, liver samples were also extracted from spontaneously delivered 1-day-old neonates, 3-day-old neonates, 5-day-old neonates, and 8-week-old adult rat. The 8-week-old adult rats were male.

The rats were kept at a constant temperature ($23 \pm 1^\circ\text{C}$) and constant humidity ($55 \pm 5\%$) at the Laboratory Animal Care and Management Facility of St. Marianna University School of Medicine. They were provided unlimited drinking water and kept at a 12-h light/12-h dark cycle. This study complied with the “Guiding principles for the care and use of laboratory animals” by The Japanese Pharmacological Society and was approved by the Experimental Animal Research Committee of St. Marianna University Graduate School of Medicine (approval number: 2002008).

2) Histological evaluation

Rat liver sections were preserved in 10% formalin, embedded in paraffin, and cut into 5- μ m sections. The sections were stained with hematoxylin and eosin. The hepatocyte size was determined as the total number of hepatocytes by area of view ($2.8 \times 10^4 \mu\text{m}^2$) under a microscope with high magnification ($\times 400$).

3) mRNA extraction and real time reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from the liver tissues using an RNeasy® sepaSol-RNA I super G kit (Nakarai, Kyoto, Japan). A NanoDrop One/OneC Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure the concentration of total RNA at an optical density of 260 nm. cDNA was synthesized using the Transcriptor First Strand cDNA Synthesis Kit (Roche Diadnostic GmbH, Mannheim, Germany). RT-PCR was performed using Step One Plus and Power SYBR® Green PCR Master Mix (Thermo Fisher Scientific). The PCR conditions were as follows: 45 cycles of denaturation at 95°C for 20 s, annealing at 60°C for 30 s, and extension at 72°C for 40 s.

Gene expression was determined using the relative values of standard curve values. In each sample, the relative value was normalized to the housekeeping gene, hypoxanthine phosphoribosyltransferase (*HPRT*). The primers used are shown in Table 1 and Table Supplement 1.

4) Immunohistochemistry

The liver samples were deparaffinized in xylene and rehydrated in graded ethanol. Endogenous peroxidase activity was inactivated with 0.1% hydrogen peroxidase. After blocking, the samples were treated with anti-albumin antibody (Proteintech, IL, USA), anti-hepatocyte growth factor (HGF) antibody (Bioss Antibodies, MA, USA), anti-hepatocyte nuclear factor 4 alpha (HNF4 α) antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and anti-thymus cell antigen 1 (Thy1) antibody (Abcam, Cambridge, UK) overnight at 4°C. A CSA Biotin-free Tyramide Signal Amplification System (Dako, Carpinteria, CA, USA) was used to demonstrate immunoreactivity according to the manufacturer's instructions. 3,3'-diaminobenzidine was used as the chromogenic substrate. The samples were then counterstained with hematoxylin.

The OLYMPUS CX41 upright microscope (Olympus, Tokyo, Japan) was used to acquire immunohistochemistry digital images. ImageJ version 1.49 software was used to calculate the density of the positive area.

5) Determination of HNF4 α levels

HNF4 α protein levels were measured using Rat HNF4A/HNF4 enzyme-linked immunosorbent assay (ELISA) Kit (LifeSpan Biosciences, WA, USA) according to the manufacturer's instructions. The tissues samples were lysed using CellLytic™ MT cell lysis reagent for mammalian tissues (Sigma-Aldrich, MO, US) and quantified total protein using Bio-Rad Protein Assay Dye Reagent Concentrate (Bio-Rad, CA, USA). Absorbance was measured at a wavelength of 450 nm using Viento nano (DS Pharma Biomedical, Osaka, Japan).

6) Statistical analysis

The results are presented as mean $\pm$ standard error of the mean. JMP pro 13 (SAS Institute Inc., Cary, NC, USA) software was used to perform statistical analyses. All mRNA expression levels were analyzed statistically using the nonparametric Steel test. The protein levels were analyzed using Dunnett's test because the sample size was small.

Results

1) Hepatocyte size

With growth, the size of the hepatocytes gradually increased from the fetuses (19-day fetuses [19F], $69.8 \pm 2.7 \mu\text{m}^2$; 21-day fetuses [21F], $145.0 \pm 13.2 \mu\text{m}^2$) to the neonates (1-day-old neonates [1N], $164.1 \pm \mu\text{m}^2$; 3-day-old neonates [3N], $162.9 \pm 5.2 \mu\text{m}^2$; 5-day-old neonates [5N], $231.0 \pm 10.2 \mu\text{m}^2$) to the adult rats ($359.1 \pm 36.0 \mu\text{m}^2$) (Fig. 1A, B). Prenatal DEX administration significantly increased the cell size in the 19F group (DEX 1.0 mg/kg, $105.7 \pm 4.95 \mu\text{m}^2$; 2.0 mg/kg, $106.4 \pm 3.64 \mu\text{m}^2$) (Fig. 1C). Lagen mRNA and mTOR levels were not affected in the 19F and 21F groups, the process of growth, or by prenatal DEX administration (Fig. S1).

2) Ki-67, cyclin B, and CDK1 mRNA levels

The mRNA expressions of proliferation and cell cycle markers Ki-67, cyclin B, and cyclin-dependent kinase 1 (CDK1) gradually decreased from 19F rats to adult rats (Fig. 2A, C, E).

The mRNA levels of cyclin B were significantly decreased in the 19F group following DEX 1.0 mg/kg administration. However, the mRNA levels of Ki-67 and CDK1 were not significantly changed. (untreated group vs. DEX 1 mg: Ki-67, 1.0 ± 0.2 vs. 0.53 ± 0.1 , $P = 0.075$; cyclin B, 1.0 ± 0.07 vs. 0.55 ± 0.09 , $P = 0.014$; and CDK1, 1.0 ± 0.07 vs. 0.74 ± 0.11 , $P = 0.17$). The mRNA levels of cyclin B decreased in the 21F group following DEX 1.0 mg/kg administration (0.56-fold) compared with that in the untreated group. The mRNA levels of CDK1 decreased in the 21F group with DEX 1.0 mg/kg (0.43-fold) and 2.0 mg/kg (0.42-fold) administration compared with that in the untreated group.

3) Thy-1 and Dlk1 expression levels

High mRNA expression levels of Thy-1 (Fig. 3A) and delta-like 1 homolog (Dlk1) (Fig. 3E) were observed in 19F rats, and the levels gradually decreased with growth. The mRNA levels of Thy-1 tended to decrease in 21F rats following DEX 1.0 mg/kg (0.43-fold) and 2.0 mg/kg (0.42-fold) administration compared with that in the untreated group; however, the mRNA levels of Dlk1 in the liver were unchanged after prenatal DEX administration (Fig. 3B, F). The number of Thy-1-protein-positive cells also decreased in 21F rats following DEX 2.0 mg/kg administration ($28.7 \pm 2.6\%$) compared with that in the untreated group ($42.3 \pm 1.4\%$) (Fig. 3C, 3D).

4) Alpha-fetoprotein (AFP) and albumin mRNA and protein levels

In the untreated group, the mRNA levels of AFP gradually decreased from 19F rats to adult rats (Fig. 4A). Although the mRNA levels of AFP were increased in 19F rats following DEX 2.0 mg/kg administration, they were unchanged in 21F rats following DEX administration (Fig. 4B). In contrast, the liver mRNA levels of albumin in 19F rats were low and gradually increased with growth (Fig. 4C, E, F). The albumin mRNA (5.79-fold) and protein levels increased in 19F rats following DEX 2.0 mg/kg administration compared with that in the untreated group (Fig. 4D, E, G).

The G6Pase and TAT mRNA levels in the livers of 8W rats were higher than those in the livers of 19F rats; however, prenatal DEX administration did not affect 19F and 21F rats (Fig.S2).

5) HGF mRNA levels and protein levels

The HGF levels increased two-fold in the 1N group compared with that in the 19F group (Fig. 5A). Administration of 1.0 mg/kg DEX in the 19F group increased 1.6-fold, and administration of 2.0 mg/kg DEX in the 21F group increased 2.9-fold compared with that in the respective untreated groups (Fig. 5B).

The immunohistochemical staining results showed that the HGF protein levels significantly increased in 19F following DEX administration (1.0 mg/kg, $34.4 \pm 1.5\%$ and 2.0 mg/kg, $25.4 \pm 1.9\%$) compared with that in the untreated group ($15.5 \pm 3.1\%$). However, the HGF protein levels were unchanged in 21F following DEX administration (Fig. 5C, D).

6) HNF4α mRNA and protein levels

HNF4α is involved in hepatocyte maturation. The HNF4α mRNA and protein levels in adult rats were higher than those in 19F rats (Fig. 6A, C).

Prenatal DEX administration significantly increased the HNF4α mRNA levels both in the 19F (DEX 1.0 mg/kg, 1.6-fold: 2.0 mg/kg, 3.7-fold) and 21F (DEX 1.0 mg/kg, 3.2-fold: 2.0 mg/kg, 2.9-fold) groups (Fig.6B).

The HNF4α protein levels were also significantly increased in the 19F (DEX 2.0 mg/kg, 51.7 ± 6.2 pg/μg) and 21F (DEX 1.0 mg/kg, 65.6 ± 2.8 pg/μg; DEX 2.0 mg/kg, 74.4 ± 12.7 pg/μg) groups following DEX administration compared with that in the untreated group (19F, 18.2 ± 3.2 pg/μg; 21F, 34.9 ± 2.7 pg/μg) (Fig.6D).

The immunohistochemical staining results showed that the HNF4α protein levels in 19F rats following DEX administration (1.0 mg/kg: $48.4 \pm 2.1\%$, 2.0 mg/kg: $42.8 \pm 1.7\%$) significantly increased compared with that in the untreated group ($34.9 \pm 2.7\%$). In addition, DEX administration significantly increased the HNF4α protein levels in 21F rats (1.0 mg/kg: $60.8 \pm 1.6\%$, 2.0 mg/kg: $65.1 \pm 2.2\%$) compared with that in the untreated group ($43.3 \pm 3.1\%$) (Fig. 6E, F).

Discussion

Preterm infants are at a risk of hepatic insufficiency because their immaturity results in a delay in achieving detoxification and synthetic functions [20].

Prenatal GC administration accelerates the development of cardiopulmonary functions for reducing respiratory distress syndrome and mortality in premature infants [21-22]. GC induces organ maturation and is necessary for sustaining extrauterine life [23]. We previously confirmed the expression of MRP2 and albumin following prenatal GC administration [24]. However, the mechanisms of fetal liver growth following prenatal GC administration remain unclear. In this study, prenatal DEX administration increased the size of fetal rat hepatocytes as well as increased the levels of maturation-related factors. This suggests that DEX induces the growth of fetal hepatocyte. We found that from 19F to 5N, the hepatocyte size gradually increased. Additionally, enlargement of hepatocytes was observed in the adult rats. Prenatal DEX administration increased the size of hepatocytes in 19F but not in 21F. Processes essential to attaining adequate liver mass and function during the fetal period include activation of specific cell lineage early in development and cell proliferation or differentiation, accomplished by enzymes and transcriptional factors. We therefore investigated whether hepatocyte enlargement depends on cell proliferation or differentiation. High mRNA levels of the cell proliferation marker Ki-67 were found in fetal hepatocytes, which gradually decreased with growth. This indicates that the capacity for cell proliferation declines with liver growth. However, prenatal DEX administration did not significantly change Ki-67 mRNA expression i.e., DEX administration may have a negligible effect on cell proliferation in fetal hepatocytes. However, other cell proliferation markers (e.g., PCNA, MCM-2, and BrdU) should be evaluated in the future. Hepatocyte proliferation and differentiation for functional growth occur simultaneously during late mammalian gestation. Gruppuso et al. (1999) reported the presence of independent signaling pathways for controlling the proliferation and differentiation in developing hepatocytes [25]. The cell cycle of hepatocytes is controlled from G(0) to mitosis by the regulation of cyclins and CDKs. Cyclin B1 forms a complex with CDK1, and the activation of this complex leads to the initiation of mitosis [26]. The mRNA expressions of cyclin B and CDK1 significantly decreased from 19F to 1N to adult rats. Additionally, prenatal DEX administration decreased the mRNA expression levels of cyclin B and CDK1 in 19F and 21F rats. The transcriptional inhibition of the cyclin B–CDK1 complex by prenatal DEX administration limited proliferation. Premature hepatocytes include hepatic progenitors during hepatocyte development [27]. Thy-1 and Dlk-1 are stem cell markers that can identify stem cell character [28, 29]. With growth, the mRNA expression levels of Thy-1 and Dlk-1 significantly decreased in the fetal liver. Prenatal DEX administration tended to decrease Thy-1 mRNA levels in 21F rats. Tanimizu et al. (2004) reported that Dlk-1 expression was strongest at murine fetal day 10.5 and was downregulated after fetal day 16.5. Because Dlk-1 expressions in the livers of 19F and 21F rats were negligible, DEX may not have had any effect [30]. The early rat fetal liver also contains Thy-1-positive cells, which gradually decrease in number with growth. The mRNA and protein levels of Thy-1 were sustained until 21F, indicating that DEX may have been influential.

Hepatic maturation is characterized according to the stage of liver growth with specific genes [31-32]. Some studies reported a high level of AFP in fetal rat hepatocytes, and the AFP level gradually decreased with hepatic maturity. In contrast, albumin and G6Pase levels were reported to be increased in mature

hepatocytes [33-34]. The mRNA levels of AFP decreased in the liver of 19F rats during growth. Our findings are in agreement with those obtained by Zhang et al. The AFP level decreased as differentiation progressed in human hepatic progenitor cells. Cells exposed to a mixture of oncostatin M, DEX, and HGF gradually featured differentiated hepatic functions, including albumin production in vitro [35]. Prenatal DEX administration did not change AFP mRNA levels in the fetal liver. The mRNA levels of albumin, TAT, and G6Pase increased in the liver from fetal rats to neonatal rats. DEX administration only increased albumin production in 19F rats. Human hepatic progenitor cells (HPCs) morphologically and functionally differentiate into hepatocytes and cholangiocytes. Freshly isolated HPCs coexpressed G6Pase, glycogen, albumin, and gamma glutamyl transpeptidase and could differentiate into functional liver cells [36]. A combination of HGF, oncostatin M, and DEX induced hepatocyte maturation. HGF in the presence of DEX induced the expression of G6Pase, TAT, and albumin in fetal hepatocytes [37]. HGF appears in the liver in the first few days after birth. We found that the mRNA and protein levels of HGF were increased in the liver of 19F rats following prenatal DEX administration. This result suggests that DEX may have a direct effect on HGF level elevation. The underlying mechanism is the subject of future research.

HNF4 α plays essential roles in structure formation and functional maturity of hepatocytes [38]. HPCs are negative for HNF4 α . They start expressing HNF4 α following their differentiation into hepatocytes [39]. The *HNF4 α* gene has two promoters of P1 and P2 isoforms in mouse liver. Transcription starts via P2 promoter during fetal life but switches to P1 at birth. Exposure to DEX in the fetal rat liver suppresses P2 but enhances the expression of transcripts from P1 [40].

The mRNA and protein levels of HNF4 α were significantly increased in the liver with growth. Prenatal DEX administration also increased their levels in the liver of 19F and 21F rats. Nyirenda et al. reported that continuous administration of prenatal DEX increased HNF4 α mRNA expression in rat livers [41]. Our results are close to clinical therapeutics and are consistent with those obtained by Nyirenda et al. Thus, an increase in hepatic HNF4 α expression is related to liver maturation.

This study did not show whether prenatal GC acts through the glucocorticoid receptor or indirectly for liver maturation. Further studies are necessary to elucidate these mechanisms using isolated fetal hepatocytes.

Conclusion

These results suggest that prenatal GC administration induces hepatocyte differentiation, and liver maturation is achieved via the expression of HNF4 α and HGF in premature fetuses.

Declarations

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Disclosure statement: To the best of our knowledge, the named authors have no conflict of interest, financial or otherwise.

Category of study: basic science

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analyzed during this study are included in this article and its supplementary information.

Code availability

Not applicable

Author' contributions

TK and YT contributed to the study conception and design. Material preparation, experiments, and data collection were performed by TK. TK, YT, YO, MO, and KK analyzed and interpreted the data. The first draft of the manuscript was prepared by TK. YT assisted in the preparation of the manuscript. MW contributed resources. NM and TI contributed to supervision and funding acquisition. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

Ethics approval

This study complied with the Guiding principles for the care and use of laboratory animals by The Japanese Pharmacological Society and was approved by the Experimental Animal Research Committee of St. Marianna University Graduate School of Medicine (approval number: 2002008).

Consent to participate

Not applicable

References

1. Sata F (2016) Developmental Origins of Health and Disease (DOHaD) and Epidemiology. *Jpn J Hyg* 71:41–46. (In Japanese) <https://doi.org/10.1265/jjh.71.41>
2. Khulan B, Liu L, Rose CM, Boyle AK, Manning JR, Drake AJ (2016) Glucocorticoids accelerate maturation of the heme pathway in fetal liver through effects on transcription and DNA methylation. *Epigenetics* 11:103–109. <https://doi.org/10.1080/15592294.2016.1144006>
3. Rog-Zielinska EA, Richardson RV, Denvir MA, Chapman KE (2014) Glucocorticoids and foetal heart maturation; implications for prematurity and foetal programming. *J Mol Endocrinol* 52:R125–R135. <https://doi.org/10.1530/JME-13-0204>
4. Hayes EJ, Paul DA, Stahl GE, Seibel-Seamon J, Dysart K, Leiby BE, Mackley AB, Berghella V (2008) Effect of antenatal corticosteroids on survival for neonates born at 23 weeks of gestation. *Obstet Gynecol* 111:921–926. <https://doi.org/10.1097/AOG.0b013e318169ce2d>
5. Sakurai K, Osada Y, Takeba Y, Mizuno M, Tsuzuki Y, Ohta Y, Ootaki M, Iri T, Aso K, Yamamoto H, Matsumoto N (2019) Exposure of immature rat heart to antenatal glucocorticoid results in cardiac proliferation. *Pediatr Int* 61:31–42. <https://doi.org/10.1111/ped.13725>
6. Mizuno M, Takeba Y, Matsumoto N, Tsuzuki Y, Asoh K, Takagi M, Kobayashi S, Yamamoto H (2010) Antenatal glucocorticoid therapy accelerates ATP production with creatine kinase increase in the growth-enhanced fetal rat heart. *Circ J* 74:171–180. <https://doi.org/10.1253/circj.cj-09-0311>
7. Tsuzuki Y, Takeba Y, Kumai T, Matsumoto N, Mizuno M, Murano K, Asoh K, Takagi M, Yamamoto H, Kobayashi S (2009) Antenatal glucocorticoid therapy increase cardiac a-enolase levels infetus and neonate rats. *Life Sci* 85:609–616. <https://doi.org/10.1016/j.lfs.2009.06.017>
8. Grijalva J, Vakili K (2013) Neonatal liver physiology. *Semin Pediatr Surg* 22:185–189. <https://doi.org/10.1053/j.sempedsurg.2013.10.006>
9. Woodgate P, Jardine LA (2015) Neonatal jaundice: phototherapy. *BMJ Clin Evid* 2015: 0319.
10. Baribault H, Marceau N (1986) Dexamethasone and dimethylsulfoxide as distinct regulators of growth and differentiation of cultured suckling rat hepatocytes. *J Cell Physiol* 129:77–84. <https://doi.org/10.1002/jcp.1041290112>
11. Monga SPS, Tang Y, Candotti F, Rashid A, Wildner O, Mishra B, Iqbal S, Mishra L (2001) Expansion of hepatic and hematopoietic stem cells utilizing mouse embryonic liver explants. *Cell Transplant* 10:81–89. <https://doi.org/10.3727/000000001783986945>
12. Snykers S, De Kock J, Rogiers V, Vanhaecke T (2009) In vitro differentiation of embryonic and adult stem cells into hepatocytes: state of the art. *Stem Cells* 27:577–605. <https://doi.org/10.1634/stemcells.2008-0963>

13. Ozdemir H, Guvenal T, Cetin M, Kaya T, Cetin A (2003) A placebo-controlled comparison of effects of repetitive doses of betamethasone and dexamethasone on lung maturation and lung, liver, and body weights of mouse pups. *Pediatr Res* 53:98–103. <https://doi.org/10.1203/00006450-200301000-00017>
14. Shiojiri N (1997) Development and differentiation of bile ducts in the mammalian liver. *Microsc Res Tech* 39:328–335. [https://doi.org/10.1002/\(SICI\)1097-0029\(19971115\)39:4<328::AID-JEMT3>3.0.CO;2-D](https://doi.org/10.1002/(SICI)1097-0029(19971115)39:4<328::AID-JEMT3>3.0.CO;2-D)
15. Kamiya A, Kinoshita T, Ito Y, Matsui T, Morikawa Y, Senba E, Nakashima K, Taga T, Yoshida K, Kishimoto T, Miyajima A (1999) Fetal liver development requires a paracrine action of oncostatin M through the gp130 signal transducer. *EMBO J* 18:2127–2136. <https://doi.org/10.1093/emboj/18.8.2127>
16. Asahina K, Fujimori H, Shimizu-Saito K, Kumashiro Y, Okamura K, Tanaka Y, Teramoto K, Arai S, Teraoka H (2004) Expression of the liver-specific gene Cyp7a1 reveals hepatic differentiation in embryoid bodies derived from mouse embryonic stem cells. *Genes Cells* 9:1297–1308. <https://doi.org/10.1111/j.1365-2443.2004.00809.x>
17. Kishi M, Emi Y, Sakaguchi M, Ikushiro S, Iyanagi T (2008) Ontogenic isoform switching of UDP-glucuronosyltransferase family 1 in rat liver. *Biochem Biophys Res Commun* 377:815–819. <https://doi.org/10.1016/j.bbrc.2008.10.043>
18. Samtani MN, Pyszczyński NA, Dubois DC, Almon RR, Jusko WJ (2006a) Modeling glucocorticoid-mediated fetal lung maturation: I. Temporal patterns of corticosteroids in rat pregnancy. *J Pharmacol Exp Ther* 317:117–126. <https://doi.org/10.1124/jpet.105.095851>
19. Samtani MN, Pyszczyński NA, Dubois DC, Almon RR, Jusko WJ (2006b) Modeling glucocorticoid-mediated fetal lung maturation: II. Temporal patterns of gene expression in fetal rat lung. *J Pharmacol Exp Ther* 317:127–138. <https://doi.org/10.1124/jpet.105.095869>
20. Bhutani VK, Wong RJ (2013) Bilirubin neurotoxicity in preterm infants: risk and prevention. *J Clin Neonatol* 2:61–69. <https://doi.org/10.4103/2249-4847.116402>
21. Wynne K, Rowe C, Delbridge M, Watkins B, Brown K, Addley J, Woods A, Murray H (2020) Antenatal corticosteroid administration for foetal lung maturation. *F1000 Faculty Rev* 219. <https://doi.org/10.12688/f1000research.20550.1>
22. Agnew EJ, Ivy JR, Stock SJ, Chapman KE (2018) Glucocorticoids, antenatal corticosteroid therapy and fetal heart maturation. *J Mol Endocrinol* 61:R61–R73. <https://doi.org/10.1530/JME-18-0077>
23. Smith LM, Qureshi N, Chao CR (2000) Effects of single and multiple courses of antenatal glucocorticoids in preterm newborns less than 30 weeks' gestation. *J Matern Fetal Med* 9:131–135. [https://doi.org/10.1002/\(SICI\)1520-6661\(200003/04\)9:2<131::AID-MFM9>3.0.CO;2-M](https://doi.org/10.1002/(SICI)1520-6661(200003/04)9:2<131::AID-MFM9>3.0.CO;2-M)
24. Takeba Y, Sakurai K, Osada Y, Mizuno M, Tsuzuki Y, Ohta Y, Ootaki M, Watanabe M, Machida T, Watanabe D, Nakamura Y, Kobayashi T, Iiri T, Naoki Matsumoto (2018) Production of bilirubin

transporters, multidrug resistance-associated protein 2 in the fetal rat liver with antenatal glucocorticoid administration. *St. Marianna Med J* 45: 281–287. (In Japanese)

25. Gruppuso PA, Bienieki TC, Faris RA (1999) The relationship between differentiation and proliferation in late gestation fetal rat hepatocytes. *Pediatr Res* 46:14–19. <https://doi.org/10.1203/00006450-199907000-00003>

26. Porter LA, Donoghue DJ (2003) Cyclin B1 and CDK1: nuclear localization and upstream regulators. *Prog Cell Cycle Res* 5:335–347.

27. Masson NM, Currie IS, Terrace JD, Garden OJ, Parks RW, Ross JA (2006) Hepatic progenitor cells in human fetal liver express the oval cell marker Thy-1. *Am J Physiol Gastrointest Liver Physiol* 291:G45–G54. <https://doi.org/10.1152/ajpgi.00465.2005>

28. Fiegel HC, Park JJ, Lioznov MV, Martin A, Jaeschke-Melli S, Kaufmann PM, Fehse B, Zander AR, Kluth D (2003) Characterization of cell types during rat liver development. *Hepatology* 37:148–154. <https://doi.org/10.1053/jhep.2003.50007>

29. Nishina H (2012) hDlk-1: a cell surface marker common to normal hepatic stem/progenitor cells and carcinomas. *J Biochem* 152:121–123. <https://doi.org/10.1093/jb/mvs069>

30. Tanimizu N, Tsujimura T, Takahide K, Kodama T, Nakamura K, Miyajima A (2004) Expression of Dlk/Pref-1 defines a subpopulation in the oval cell compartment of rat liver. *Gene Expr Patterns* 5:209–218. <https://doi.org/10.1016/j.modgep.2004.08.003>

31. Derman E, Krauter K, Walling L, Weinberger C, Ray M, Darnell JE Jr. (1981) Transcriptional control in the production of liver-specific mRNAs. *Cell* 23:731–739. [https://doi.org/10.1016/0092-8674\(81\)90436-0](https://doi.org/10.1016/0092-8674(81)90436-0)

32. Panduro A, F Shalaby, D A Shafritz (1987) Changing patterns of transcriptional and post-transcriptional control of liver-specific gene expression during rat development. *Genes Dev* 1:1172–1182. <https://doi.org/10.1101/gad.1.10.1172>

33. Jochheim A, Hillemann T, Kania G, Scharf J, Attaran M, Manns MP, Wobus AM, Ott M (2004) Quantitative gene expression profiling reveals a fetal hepatic phenotype of murine ES-derived hepatocytes. *Int J Dev Biol* 48:23–29. <https://doi.org/10.1387/ijdb.15005571>

34. Sell S (2008) Alpha-fetoprotein, stem cells and cancer: how study of the production of alpha-fetoprotein during chemical hepatocarcinogenesis led to reaffirmation of the stem cell theory of cancer. *Tumour Biol* 29:161–180. <https://doi.org/10.1159/000143402>

35. Zhang W, Li W, Liu B, Wang P, Li W, Zhang H (2012) Efficient generation of functional hepatocyte-like cells from human fetal hepatic progenitor cells in vitro. *J Cell Physiol* 227:2051–2058. <https://doi.org/10.1002/jcp.22934>

36. Nava S, Westgren M, Jaksch M, Tibell A, Broomé U, Ericzon, BG, Sumitran-Holgersson S (2005) Characterization of cells in the developing human liver. *Differentiation* 73:249–260. <https://doi.org/10.1111/j.1432-0436.2005.00019.x>
37. Kamiya A, Kinoshita T, Miyajima A (2001) Oncostatin M and hepatocyte growth factor induce hepatic maturation via distinct signaling pathways. *FEBS Lett* 492:90-94. [https://doi.org/10.1016/s0014-5793\(01\)02140-8](https://doi.org/10.1016/s0014-5793(01)02140-8)
38. Li J, Ning G, Duncan SA (2000) Mammalian hepatocyte differentiation requires the transcription factor HNF-4alpha. *Genes Dev* 14:464–474. <https://doi.org/10.1101/gad.14.4.464>
39. Parviz F, Matullo C, Garrison WD, Savatski L, Adamson JW, Ning G, Kaestner KH, Rossi JM, Zaret KS, Duncan SA (2003) Hepatocyte nuclear factor 4alpha controls the development of a hepatic epithelium and liver morphogenesis. *Nat Genet* 34:292–296. <https://doi.org/10.1038/ng1175>
40. Dean S, Tang JI, Seckl JR, Nyirenda MJ (2010) Developmental and tissue-specific regulation of hepatocyte nuclear factor 4-alpha (HNF4-alpha) isoforms in rodents. *Gene Expr* 14:337–344. <https://doi.org/10.3727/105221610x12717040569901>
41. Nyirenda MJ, Dean S, Lyons V, Chapman KE, Seckl JR (2006) Prenatal programming of hepatocyte nuclear factor 4alpha in the rat: A key mechanism in the 'foetal origins of hyperglycaemia'? *Diabetologia* 49:1412–1420. <https://doi.org/10.1007/s00125-006-0188-5>

Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

Figure 1

Figure 1

Histology of hepatocytes in liver tissue with growth (A) Histology of hepatocytes with hematoxylin and eosin staining in day-19 fetuses (19F), day-21 fetuses (21F), 1-day-old (1N) rats, 3-day-old (3N) rats, 5-day-old (5N) rats, and 8-weeks adult rats (8W) in liver tissue with growth and in 19F and 21F rats after prenatal DEX administration. The photos show images at high magnification ($\times 400$), scale bar = 20 μm . The field of view of $2.8 \times 10^4 \mu\text{m}^2$ was divided by total hepatocyte number to obtain the hepatocyte size

in liver tissues with growth (B) and in the liver of fetal rats after DEX administration. Fetal liver, neonatal liver, and adult rat liver tissues: n = 3. Two nonadjacent locations were photographed, for each tissue specimen (C). *P < 0.05, **P < 0.01, and ***P < 0.001 vs. 19F group. †††P < 0.001 vs the respective untreated group.

Figure 2

Figure 2

Ki-67, cyclin B, and CDK1 mRNA and protein levels Ki-67, cyclin B, and CDK1 mRNA levels were determined in liver tissues in 19F and 8W rats (A), (C), and (E). Ki-67, cyclin B, and CDK1 mRNA levels were determined in fetal rats after prenatal DEX administration (B), (D), and (F). Fetal liver, neonatal liver, and adult rat liver samples (mRNA): n = 7. *P < 0.05 and **P < 0.01 vs. the respective 19 fetus group. †P < 0.05 and ††P < 0.01 vs. the respective untreated group.

Figure 3

Figure 3

Thy1 and Dlk1 mRNA and protein levels Thy-1 and Dlk1 mRNA levels were determined in liver tissues in 19F and 8W rats (A) and (E). Thy-1 and Dlk1 mRNA levels were determined in 19F and 21F rats after prenatal DEX administration (B) and (F). Fetal, neonatal, and adult rat mRNA samples: n = 7. In immunohistochemistry, images of Thy-1 production in liver tissue after prenatal DEX administration (C). Thy-1 positive staining intensity was analyzed using NIH image analysis. 19F rat tissues specimens: n = 4. 21F, 1N, and 8W rat tissues specimens: n = 3. Two nonadjacent locations were photographed for each tissue specimen (D). **P < 0.01 vs. the respective 19-day fetus group. †P < 0.05 vs. the respective untreated group.

Figure 4

Figure 4

AFP mRNA and albumin mRNA and protein levels AFP and albumin mRNA levels were determined in liver tissues from 19F to 8W (A) and (C). AFP and albumin mRNA levels were determined in 19F and 21F rats after prenatal DEX administration (B) and (D). Fetal, neonatal, and adult rat mRNA samples: n = 7. In immunohistochemistry, albumin production increased in liver tissue after prenatal DEX administration (E). Albumin positive staining intensity was analyzed using NIH image analysis. 19F rat tissues

specimens: n = 4. 21F, 1N, and adult rat tissues specimens: n = 3. Two nonadjacent locations were photographed, for each tissue specimen (F) and (G). *P < 0.05, **P < 0.01, and ***P < 0.001 vs. the respective 19 fetus group. †P < 0.05 and ††P < 0.01 vs the respective untreated group.

Figure 5

Figure 5

HGF mRNA and protein levels HGF mRNA levels were determined in liver tissues from 19F to 8W (A). HGF mRNA levels were determined in 19F and 21F rats after prenatal DEX administration (B). Fetal, neonatal,

and adult rat mRNA samples: n = 7. In immunohistochemistry, HGF production increased in liver tissue of 19F after prenatal DEX administration (C). HGF positive staining intensity was analyzed using NIH image analysis. 19F rat tissues specimens: n = 4. 21F, 1N, and 8W rat tissues specimens: n = 3. Two nonadjacent locations were photographed for each tissue specimen (D). *P < 0.05 vs. the respective 19 fetus group. †P < 0.05, ††P < 0.01, and †††P < 0.001 vs. the respective untreated group.

Figure 6

Figure 6

HNF4α mRNA and protein levels. HNF4α mRNA levels were determined in liver tissues from 19F to 8W (A). HNF4α mRNA levels were determined in 19F and 21F rats after prenatal DEX administration (B). Fetal, neonatal, and adult rat mRNA samples: n = 7. ELISA of HNF4α protein production in liver tissue after prenatal DEX administration (C) and (D). Fetal, neonatal, and adult rat protein extract samples: n = 4. In immunohistochemistry, albumin production was analyzed in liver tissue after prenatal DEX (E). HNF4α and albumin positive staining intensity were analyzed using NIH image analysis. 19F rat tissues specimens: n = 4. 21F, 1N, 8W rat tissues specimens: n = 3. Two nonadjacent locations were photographed for each tissue specimen (F). **P < 0.01 and ***P < 0.001 vs. the respective 19 fetus group. †P < 0.05, ††P < 0.01 and †††P < 0.001 vs the respective untreated group

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