ORIGINAL

Role of Nrf2 signaling in development of hepatocyte-like cells

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Abstract : Generation of hepatocytes from human adipose-derived mesenchymal stem cells (hADSCs) could be a promising alternative source of human hepatocytes. However, mechanisms to differentiate hepatocytes from hADSCs are not fully elucidated. We have previously demonstrated that our three-step differentiation protocol with glycogen synthase kinase (GSK) 3 inhibitor was effective to improve hepatocyte functions. In this study, we investigated the activation of the nuclear factor erythroid-2 related factor 2 (Nrf2) on hADSCs undergoing differentiation to HLC (hepatocyte-like cells). Our three-step differentiation protocol was applied for 21 days (Step 1 : day 1-6, Step2 : day 6-11, Step3 : day 11-21). Our results show that significant nuclear translocation of Nrf2 occurred from day 11 until the end of HLC differentiation. Nuclear translocation of Nrf2 and CYP3A4 activity in the GSK3 inhibitor-treated group was obviously higher than that in Activin A-treated groups at day 11. The maturation of HLCs was delayed in Nrf2-siRNA group compared to control group. Furthermore, CYP3A4 activity in Nrf2-siRNA group was decreased at the almost same level in Activin A-treated group. Nrf2 translocation might enhance the function of HLC and be a target for developing highly functional HLC. J. Med. Invest. 70 : 343-349, August, 2023

Keywords **:** *HLC, ADSC, differentiation*

INTRODUCTION

Globally, more than 1 million people die of liver disease every year (1, 2). For acute or chronic liver failure associated with end-stage liver dysfunction, liver transplantation is regarded as the only available therapy. However, considering the lack of healthy donors, hepatocyte transplantation shows potential as an alternative treatment – except for liver transplantation (3-5). The demand for liver transplantation has significantly outstripped the supply of donor organs. Consequently, multiple attempts to expand the availability of donor organs have been employed, including opt-out organ donation programs, use of suboptimal donor organs [e.g. from deceased cardiac donors or steatotic (fatty) livers], split-donor transplantation, and living-donor liver transplantation (6, 7). Fortunately, unlike most other organs, the introduction of exogenous hepatocytes into the liver parenchyma is a relatively simple undertaking, suggesting that the liver is highly amenable to tissue therapy using induced pluripotent stem cell–derived hepatocytes (8, 9). Several studies showed that human mesenchymal stem cells (MSCs) derived from adipose tissue, bone marrow, or umbilical cord blood are capable of differentiating into hepatocyte-like cells (HLCs) in specialized *in vitro* culture conditions (10-14). Recent studies also demonstrated that hepatocytes derived from human adipose stem cells (hADSCs) are a potentially scalable and applicable alternative to human hepatocytes (13, 15-18). However, a precise mechanism to generate mature HLCs from hADSCs remains unclear.

The nuclear factor erythroid-2 related factor 2 (Nrf2)/Kelch-like ECH-associated protein 1 (Keap1) pathway protects cells against oxidative stress. In response to oxidative stress, Nrf2 dissociates from Keap1 and enters the nucleus, whereby it regulates transcription of hemeoxygenase-1 and other target genes encoding proteins for detoxification, energy metabolism, or other cytoprotective functions (19-23). For example, activated Nrf2 signaling reportedly plays a protective role in liver injury associated with nonalcoholic steatohepatitis, and overexpression of hepatic Nrf2 can functionally restore liver injury in mouse models (24). Furthermore, the Nrf2/farnesoid X receptor/liver X receptor pathway was found to regulate lipogenesis and CYP3A4 expression in murine hepatocytes (25). Here, we investigated the involvement of Nrf2 in differentiation of HLCs from hADSCs.

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MATERIALS AND METHODS

Culture and differentiation of hADSCs

For this study, we applied our established three-step differentiation protocol, as previously reported (26). Briefly, hADSCs (StemPro Human Adipose-Derived Stem Cells, R7788-115) purchased from Invitrogen (Waltham, MA, USA) and cultured in MesenPRO RS medium (Thermo Fisher Scientific, USA). hADSCs were passaged three times before use in our three-step differentiation protocol.

In Step 1, definitive endoderm differentiation was induced by incubating cells with Dulbecco's Modified Eagle's Medium with F-12 Supplement containing 0.5 mg/mL bovine serum albumin fraction V (BSA ; Sigma-Aldrich, Tokyo, Japan) and 2 μM CHIR99021 [glycogen synthase kinase 3 (GSK3) inhibitor ; Selleck Chemicals, Houston, TX, USA) for 24 h from day 3. The following day, 1% insulin-transferrin-selenium (ITS, Sigma-Aldrich) was added to the medium and cells were cultured for 2 days. In Step 2, subsequent hepatoblast differentiation was promoted by changing the medium to Minimum Essential Medium with non-essential amino acids (Thermo Scientific) supplemented with 0.5 mg/mL BSA, 1% ITS, 20 ng/mL bone morphogenic protein 2 (PeproTech, Rocky Hill, NJ, USA), and 30 ng/mL fibroblast growth factor 4 (FGF4, PeproTech) for 5 days. In Step 3, hepatocyte differentiation is stimulated by treating cells with 20 ng/mL hepatocyte growth factor (HGF, PeproTech)

for 5 days, followed by 20 ng/mL HGF, 10 ng/mL oncostatin M (PeproTech), and 1×10^{-6} M dexamethasone (Sigma-Aldrich) for another 5 days.

In the Nrf2-knockdown group, hADSCs were transfected with an Nrf2 siRNA (10 nmol/L, S9492 ; Applied Biosystems, Waltham, MA, USA) using Lipofectamine RNAiMAX Transfection Reagent (Invitrogen) according to the manufacturer's instructions.

Immunofluorescence

We cultured the cells in chamber slides (Matsunami, Lot No. 191029) for immunofluorescence. Next, we removed the covers of chamber slides, washed the cells in the bottom with phosphate-buffered saline (PBS, 4°C), and fixed slides with 4% paraformaldehyde. After washing with PBS, slides were treated with Triton X-100 (Lot No. 308T1683 ; Kanto Chemical Co., Tokyo, Japan), washed again, and incubated with an anti-Nrf2 primary antibody (ab62352 ; Abcam, Cambridge, UK) overnight at 4°C. The next day, slides were washed and incubated with a fluorophore-conjugated secondary antibody (A21428 ; Life Technologies, Waltham, MA, USA), and sealed with ProLong Gold Antifade Reagent with DAPI (P36931 ; Thermo Fisher Scientific, MA, USA). Finally, slides were observed and imaged with a fluorescence microscope (Keyence Corporation, Itasca, IL, USA). Nrf2 translocation was defined when the color showed yellow (blue ; nucleus, red ; Nrf2, yellow : merged red and green) (27-29). The translocation ratio of Nrf2 was obtained from the number of Nrf2 nuclear stained cells (yellow) divided by the number of DAPI stained cells (DAPI) in five independent ×40 magnification.

RNA isolation and real-time PCR (RT-PCR)

Total RNA was isolated with a RNeasy Mini Kit (Qiagen, Hilden, Germany) and subsequently synthesized into complementary DNA with a reverse transcription kit (Applied Biosystems). RT-PCR was performed in triplicate using a StepOnePlus Real-Time PCR System (Applied Biosystems). Human *GAPDH* (4352339E, Applied Biosystems) was used as an internal control.

CYP3A4 activity assay

CYP3A4 enzyme activity was assessed by a P450-Glo assay (Promega, Madison, Wisconsin) in accordance with the manufacturer's instructions. Luminescence was measured with a luminometer (2104-0010 ; Envision, Waltham, Massachusetts).

Urea Assay

Culture supernatants of HLCs from each step were isolated and measured using a urea nitrogen kit (BioAssay Systems, San Francisco, CA, USA) to quantify urea production. Optical density values of sample plate wells were detected with a plate reader.

Statistical analysis

Data analysis was performed with GraphPad Prism 5 software, which was used to conduct one-way ANOVA and Mann–Whitney U tests. All data are presented as mean ± standard deviation. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Differentiation of HLCs from hADSCs

First, we differentiated hADSCs to HLCs according to our established three-step protocol (26) (Fig. 1A). The morphology of HLCs changed during each step ; eventually, HLCs adopted a round shape compared with the long, spindle-like shape of hADSCs (Fig. 1B). We next evaluated expression of relevant genes to confirm the HLC differentiation. Our results confirmed significant decreases of stemness markers such as SOX (SRY-Box Transcription Factor) 2 ($p < 0.001$, Fig. 2A) and OCT4 (octamer-binding transcription factor 4) ($p < 0.01$ Fig. 2B), as well as significant increases of the expression of endoderm-specific genes such as SOX17 ($p < 0.001$, Fig. 2C) and CXCR4 (C-X-C Chemokine Receptor Type 4) ($p < 0.001$, Fig. 2D) at day 6 (end of Step 1). Next, the elevation of hepatocyte-related gene AFP, a hepatoblast marker, was confirmed during Step 2 (Fig. 2C). Finally, expression of the hepatocyte gene ALB, a marker of hepatocyte maturation, was confirmed during Step 3 (Fig. 2D).

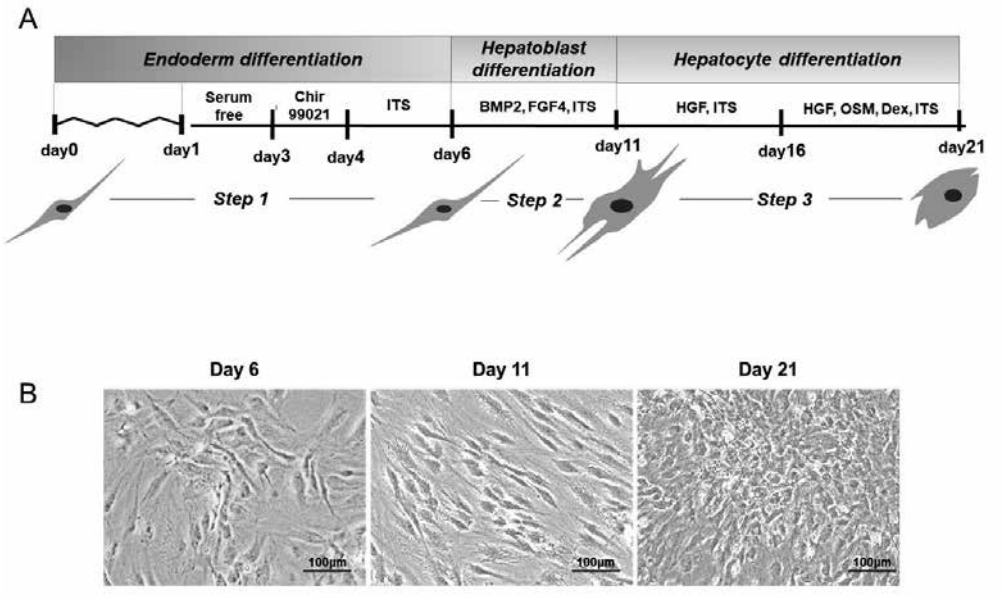

Figure 1. Differentiation of HLCs from hADSCs.
(A) Three-step protocol for differentiation of HLCs. (B) Morphology of HLCs at day 6, day 11, and day 21.

Gene expression and hepatocyte functions

Expression of genes indicative of the ammonium clearance function of hepatocytes, namely CPS1 (Carbamoyl phosphate synthase 1) ($p < 0.001$, Fig. 3A), SLC25A1 (Fig. 3B), and AAT (alpha-1 antitrypsin) ($p < 0.05$, Fig. 3C), were notably increased in HLCs compared with hADSCs, although there was still a great distance to reach levels observed in primary hepatocytes. Similar results were observed for CYP3A4 activity, as indicated by significantly increased expression of Luciferin-IPA in HLCs at day 21 ($p < 0.001$, Fig. 3D). Urea synthesis was also higher in HLCs at Day 21 compared with hADSCs ($p < 0.001$, Fig. 3E) but lower than primary hepatocytes.

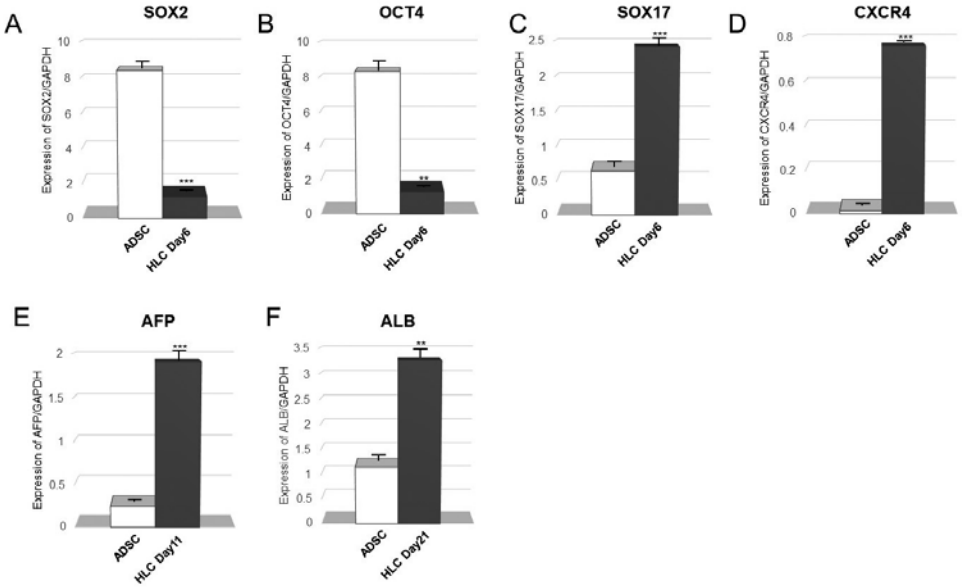

Figure 2. Hepatic-specific gene expression during three-step differentiation of hADSCs into HLCs.
Step 1 : mRNA expression of SOX2 (A), OCT4 (B), SOX17 (C), and CXCR4 (D) in HLCs at day 6.
Step 2 : mRNA expression of AFP, a hepatoblast marker, in HLCs at day 11 (E).
Step 3 : mRNA expression of ALB, a hepatocyte maturation marker, in HLCs day 21 (F).
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

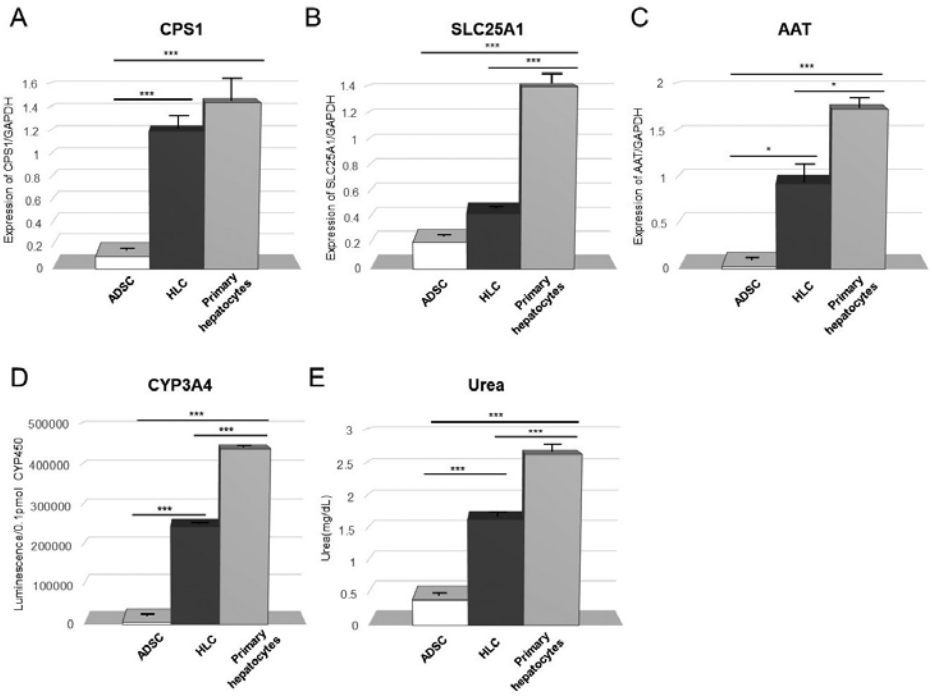

Figure 3. Expression of hepatic-specific genes in HLCs.
mRNA expression of (A) CPS1, (B) SLC25A1, and (C) AAT in HLCs at day 21. (D) CYP3A4 activity at day 21. (E) Urea Assay of HLCs at day 21. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Nuclear translocation of Nrf2 increased during differentiation of HLCs

We assessed the distribution of Nrf2 (cytoplasmic or nuclear) during the 21 days of differentiation by immunofluorescence (Fig. 4A). Nuclear translocation of Nrf2 was not observed at day 6 (end of Step 1) during definitive endoderm differentiation. However, nuclear translocation of Nrf2 was notably increased at during hepatoblast differentiation compared to Step 1 (Fig. 4B, $P < 0.001$). The rate of nuclear translocation of Nrf2 was still highly observed at day21 (Step 3) but there was no difference between Step 2 and Step 3.

Nuclear translocation of Nrf2 in the presence of Activin A or GSK3 inhibitor

Our protocol used CHIR99021 (GSK3 inhibitor) to induce definitive endoderm differentiation during Step 1. We also investigated the effect of Activin A, a multifunctional cytokine, that plays important roles in the microenvironment of hepatocyte phenotypes and regional endoderm specification (1). Activin A was generally used for definitive endoderm differentiation during Step 1. Nuclear translocation of Nrf2 was investigated among the three groups : untreated hADSCs, GSK3 inhibitor-treated hADSCs, Activin A-treated hADSCs. No differences in nuclear translocation of Nrf2 were observed between the three groups at day 6 (end of Step 1 ; Fig. 5A). Although nuclear translocation of Nrf2 was observed in Activin A-treated cells, it was significantly higher in the GSK3 inhibitor-treated group compared to untreated hADSCs ($p < 0.001$) and Activin A-treated hADSCs ($p < 0.001$) at day 11 (end of Step 2 ; Fig. 5B and 5D). Nuclear translocation of Nrf2 in the GSK3 inhibitor-treated group was still significantly higher than the other three groups (Fig. 5C and 5E, $p < 0.001$).

Effect of knockdown of Nrf2 in differentiation of HLC

We next knocked down Nrf2 expression during Step 1 with siRNA. The results of morphological analyses showed that cells in the siRNA-treated group at day 21 were not completely differentiated into HLCs (Fig. 6A). Nrf2-siRNA treated hADSCs at day 21 did not have a spindle shape and had spindle form. Functional comparison of HLCs with a CYP3A4 activity assay (Luciferin-IPA) revealed that the GSK3 inhibitor-treated group had significantly higher activity than the other three groups ($p < 0.05$) (Fig. 6B). However, this result was cancelled by the addition of siRNA Nrf2. CYP3A4 activity in Nrf2-siRNA treated hADSCs was almost same in that in Activin A-treated hADSCs.

DISCUSSION

In the present study, we established human HLCs derived from hADSCs according to a three-step protocol (15). Nrf2 was activated during the procedure, and the maturation of HLCs was delayed in Nrf2-siRNA group. Thus, Nrf2 might be a target for developing highly functional human HLCs.

Generation of HLCs derived from hADSCs could be a promising alternative source of human hepatocytes. Of the donor sources of hepatocytes proposed, including induced pluripotent stem cells, embryonic stem cells, and MSCs, MSCs may be best cell source because they can overcome problems associated with genetic damage, rejection, and ethics. In particular, ADSCs – a type of MSC derived from fat tissues – can be collected from patients with minimal invasiveness. We previously reported the effectiveness of a three-dimensional culture system for HLCs generated from hADSCs with an recombinant peptide μ-piece (26). However, mechanisms underlying the differentiation of hepatocytes from hADSCs are not well elucidated.

We previously reported that Nrf2, a transcription factor that regulates cellular defenses against oxidative stress (30), protects against liver ischemia/reperfusion injury (31) and calcineurin inhibitor-induced nephrotoxicity (32) by inhibiting oxidative stress, inflammation, and apoptosis. In addition, Nrf2 maintains optimal levels of intracellular reactive oxygens species to regulate adipocyte differentiation (33). Moreover, Nrf2 overexpression in MSCs induce stem-cell marker expression and enhances osteoblastic differentiation by preventing apoptosis under oxidative stress (34). Considering this perspective, we anticipated a role for Nrf2 in regulating hADSC differentiation.

Wnt signaling is key for the differentiation of MSCs (35). Wnt/β-catenin signaling regulates foregut endoderm fate, proliferation, and morphogenesis (36). Previous studies revealed that Wnt signaling is required to specify definitive endoderm from hADSCs, and manipulations of Wnt signaling through use of GSK3 inhibitors have been applied to direct differentiation of definitive endoderm and hepatocytes (37). GSK3 inhibitors upregulate transcription factors involved in specification of

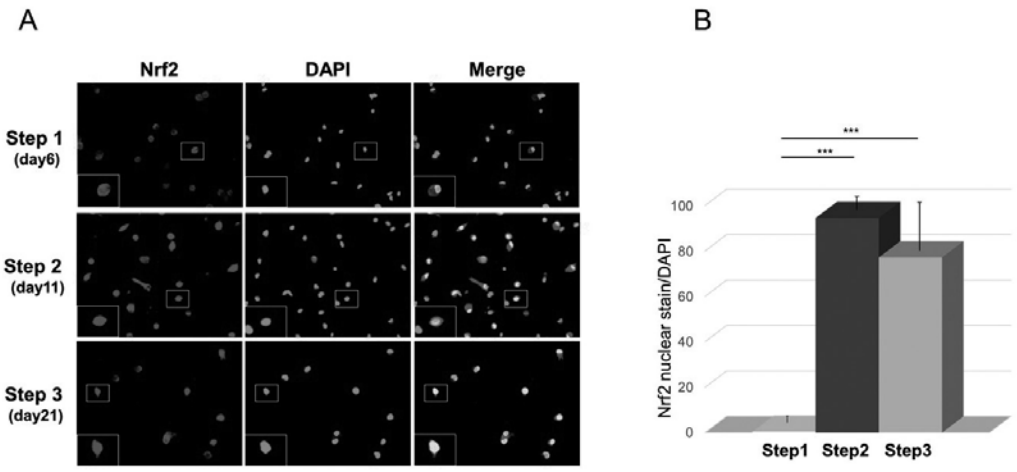

Figure 4. Nuclear translocation of Nrf2 in each step.
(A) Immunofluorescence staining of Nrf2 expression in HLCs at each step. (B) Quantification and graphical presentation of the Nrf2 translocation rates. *** $P < 0.001$.

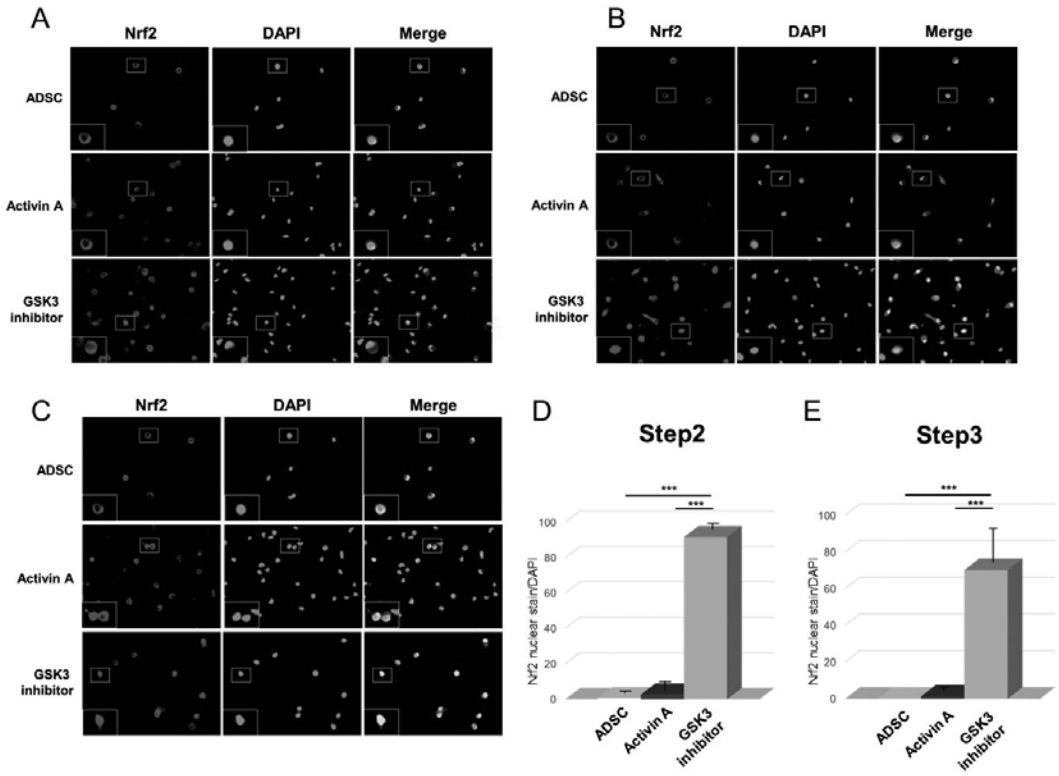

Figure 5. Nuclear translocation of Nrf2 in each group.
Immunofluorescence staining of Nrf2 expression following treatment with GSK3 inhibitor, Activin A, or GSK3 inhibitor with Nrf2 siRNA at day 6 (A), day 11 (B), and day 21 (C). Quantification and graphical presentation of the Nrf2 translocation rates at day 11 (D) and day 21 (E). *** $P < 0.001$.

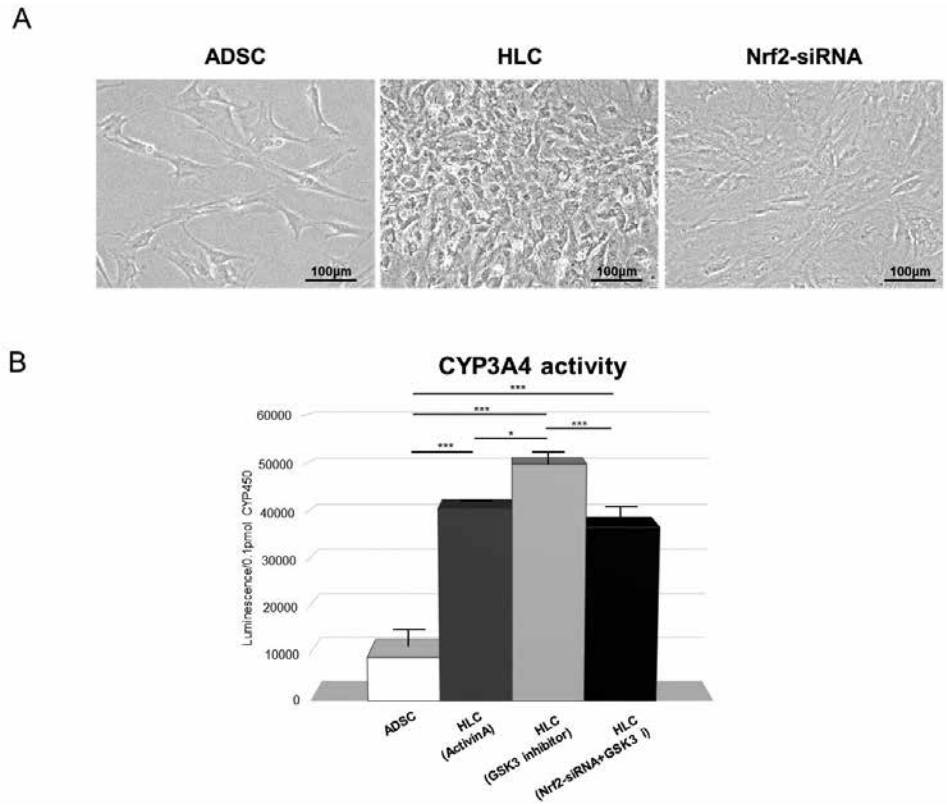

Figure 6. Morphology of HLCs in siRNA-Nrf2.
(A) Morphology of HLCs at day 21 (B) CYP3A4 activity was highest in the GSK3 inhibitor-treated group. However, this effect was cancelled by Nrf2 siRNA. * $P < 0.05$, ** P value < 0.01, *** $P < 0.001$.

definitive endoderm, namely GATA4 (GATA Binding Protein 4), FOXA2 (Forkhead Box A2), SOX17, and CXCR4 (15). In this study, we used the GSK3 inhibitor (CHIR99021) in Step 1 and successfully acquired homogenous hepatic progenitor cells. Notably, GSK3β is a negative regulator of Nrf2 (38). Exposure of cells to inducers of antioxidant response elements (AREs), such as oxidative stress, leads to the dissociation of Nrf2 from its cytosolic inhibitor Keap1 in a process mediated by protein kinase C (38). After nuclear translocation, Nrf2 promotes the AREs of many cytoprotective genes in combination with other transcription factors, such as small musculoaponeurotic fibrosarcoma, activation transcription factor 4, and polyamine-modulated factor 1. Nuclear export of Nrf2 is regulated by the tyrosine kinase Fyn. Moreover, GSK3β acts upstream of Fyn kinase to control nuclear export and subsequent proteasomal degradation of Nrf2. Because a previous report showed an equal ability of CHIR99021 and Activin A to induce hepatic progenitor cells (15), the role of Nrf2 seems to be not vital for endoderm differentiation. In line with this notion, application of the GSK3 inhibitor did not significantly enhance Nrf2 nuclear translocation during Step 1 in the present study compared with Activin A. However, knockdown of Nrf2 during Step 1 resulted in a longer time for HLCs to mature in the present study, implicating the importance of the Nrf2 pathway in promoting HLC differentiation. Moreover, rates of Nrf2 nuclear translocation were higher in the presence of the GSK3 inhibitor compared with Activin A in Step 2. Thus, Nrf2 may play an important role in hepatoblast differentiation not in definitive endoderm differentiation.

Another possible mechanism is FGFR4-GSK3β signaling, which promotes translocation of Nrf2 to the nucleus. FGFR4 receptors on the surface of hepatocellular carcinoma cells were activated by FGF19 through inactivation of GSK3β in the tumor microenvironment (39). Although it the role of the FGFR4-GSK3β-Nrf2 pathway under basal conditions is unknown, the homodimer of FGFR4 binds with FGF subfamily members including FGF4 or FGF19. We used FGF4 for hepatoblast differentiation in Step 2. FGF4 is the key cytokine required for differentiation of MSCs into a hepatic lineage (40). Nuclear translocation of Nrf2 mainly occurred during Step 2. Taken together, the FGFR4-GSK3β-Nrf2 pathway may play a key role in hepatoblast differentiation, although further investigation is needed.

As a limitation of the present study, we did not investigate other molecules in the Wnt signaling pathway or GFR4-GSK3β-Nrf2 pathway. Second, we did not investigate the effect of a specific Nrf2 inducer, such as dimethyl fumarate. Furthermore, only single siRNA was used in this study. Third, we did not perform transplantation of HLCs in this study to verify their actual function *in vivo*.

In conclusion, we demonstrated that Nrf2 was activated during differentiation of HLCs, especially during the hepatoblast differentiation step, whereas knockdown of Nrf2 might delay the maturation and impaired specific functions of HLCs. Our findings suggest that Nrf2 is a target for developing highly functional human HLCs.

CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

REFERENCES

1. Gonzalez SA, Keeffe EB : Chronic viral hepatitis : epidemiology, molecular biology, and antiviral therapy. Front Biosci (Landmark Ed) 16 : 225-250, 2011
2. Hou J, Long Y, Hu B, Huang S, Xu G, Gao T, Wu F, Li Y, Wu ZK : Retaining mTeSR1 Medium during Hepatic Differentiation Facilitates Hepatocyte-Like Cell Survival by Decreasing Apoptosis. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology 51 : 1533-1543, 2018
3. Asumda FZ, Hatzistergos KE, Dykxhoorn DM, Jakubski S, Edwards J, Thomas E, Schiff ER : Differentiation of hepatocyte-like cells from human pluripotent stem cells using small molecules. Differentiation ; research in biological diversity 101 : 16-24, 2018
4. Schwartz RE, Bram Y, Frankel A : Pluripotent Stem Cell-Derived Hepatocyte-like Cells : A Tool to Study Infectious Disease. Current pathobiology reports 4 : 147-156, 2016
5. Takayama K, Hagihara Y, Toba Y, Sekiguchi K, Sakurai F, Mizuguchi H : Enrichment of high-functioning human iPS cell-derived hepatocyte-like cells for pharmaceutical research. Biomaterials 161 : 24-32, 2018
6. Starzl TE, Groth CG, Brettschneider L, Penn I, Fulginiti VA, Moon JB, Blanchard H, Martin AJ Jr, Porter KA : Orthotopic homotransplantation of the human liver. Annals of surgery 168 : 392-415, 1968
7. Starzl TE, Fung JJ : Themes of liver transplantation. Hepatology 51 : 1869-1884, 2010
8. Si-Tayeb K, Noto FK, Nagaoka M, Li J, Battle MA, Duris C, North PE, Dalton S, Duncan SA : Highly efficient generation of human hepatocyte-like cells from induced pluripotent stem cells. Hepatology 51 : 297-305, 2010
9. Fisher RA, Strom SC : Human hepatocyte transplantation: worldwide results. Transplantation 82 : 441-449, 2006
10. Sa-Ngiamsuntorn K, Hongeng S, Wongkajornsilp A : Development of Hepatocyte-like Cell Derived from Human Induced Pluripotent Stem cell as a Host for Clinically Isolated Hepatitis C Virus. Current protocols in stem cell biology 42 : 4A 13 11-14A 13 34, 2017
11. Yamamoto Y, Banas A, Murata S, Ishikawa M, Lim CR, Teratani T, Hatada I, Matsubara K, Kato T, Ochiya T : A comparative analysis of the transcriptome and signal pathways in hepatic differentiation of human adipose mesenchymal stem cells. The FEBS journal 275 : 1260-1273, 2008
12. Pongjantarasatian S, Kadegasem P, Sasanakul W, Sa-Ngiamsuntorn K, Borwornpinyo S, Sirachainan N, Chuansumrit A, Tanratana P, Hongeng S : Coagulant activity of recombinant human factor VII produced by lentiviral human F7 gene transfer in immortalized hepatocyte-like cell line. PloS one 14 : e0220825, 2019
13. Banas A, Teratani T, Yamamoto Y, Tokuhara M, Takeshita F, Quinn G, Okochi H, Ochiya T : Adipose tissue-derived mesenchymal stem cells as a source of human hepatocytes. Hepatology 46 : 219-228, 2007
14. Zemel R, Bachmetov L, Ad-El D, Abraham A, Tur-Kaspa R : Expression of liver-specific markers in naive adipose-derived mesenchymal stem cells. Liver international : official journal of the International Association for the Study of the Liver 29 : 1326-1337, 2009
15. Huang J, Guo X, Li W, Zhang H : Activation of Wnt/beta-catenin signalling via GSK3 inhibitors direct differentiation of human adipose stem cells into functional hepatocytes. Scientific reports 7 : 40716, 2017
16. D'Souza N, Rossignoli F, Golinelli G, Grisendi G, Spano C, Candini O, Osturu S, Catani F, Paolucci P, Horwitz EM, Dominici M : Mesenchymal stem/stromal cells as a delivery platform in cell and gene therapies. BMC medicine 13 : 186, 2015

17. Clynes M : Cell culture models for study of differentiated adipose cells. Stem cell research & therapy 5 : 137, 2014
18. Xu D, Nishimura T, Zheng M, Wu M, Su H, Sato N, Lee G, Michie S, Glenn J, Peltz G : Enabling autologous human liver regeneration with differentiated adipocyte stem cells. Cell transplantation 23 : 1573-1584, 2014
19. McLoughlin MR, Orlicky DJ, Prigge JR, Krishna P, Talago EA, Cavigli IR, Eriksson S, Miller CG, Kundert JA, Sayin VI, Sabol RA, Heinemann J, Brandenberger LO, Iverson SV, Bothner B, Papagiannakopoulos T, Shearn CT, Arner ESJ, Schmidt EE : TrxR1, Gsr, and oxidative stress determine hepatocellular carcinoma malignancy. Proceedings of the National Academy of Sciences of the United States of America 116 : 11408-11417, 2019
20. Ma Q : Role of nrf2 in oxidative stress and toxicity. Annual review of pharmacology and toxicology 53, 401-426, 2013
21. Kansanen E, Kuosmanen SM, Leinonen H, Levonen AL : The Keap1-Nrf2 pathway : Mechanisms of activation and dysregulation in cancer. Redox biology 1 : 45-49, 2013
22. Motohashi H, Yamamoto M : Nrf2-Keap1 defines a physiologically important stress response mechanism. Trends in molecular medicine 10 : 549-557, 2004
23. Okawa H, Motohashi H, Kobayashi A, Aburatani H, Kensler TW, Yamamoto M : Hepatocyte-specific deletion of the keap1 gene activates Nrf2 and confers potent resistance against acute drug toxicity. Biochemical and biophysical research communications 339 : 79-88, 2006
24. Chen X, Xue H, Fang W, Chen K, Chen S, Yang W, Shen T, Zhang P, Ling W : Adropin protects against liver injury in nonalcoholic steatohepatitis via the Nrf2 mediated antioxidant capacity. Redox biology 21 : 101068, 2019
25. Yan C, Zhang Y, Zhang X, Aa J, Wang G, Xie Y : Curcumin regulates endogenous and exogenous metabolism via Nrf2-FXR-LXR pathway in NAFLD mice. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 105 : 274-281, 2018
26. Saito Y, Ikemoto T, Tokuda K, Miyazaki K, Yamada S, Imura S, Miyake M, Morine Y, Oyadomari S, Shimada, M : Effective three-dimensional culture of hepatocyte-like cells generated from human adipose-derived mesenchymal stem cells. Journal of hepato-biliary-pancreatic sciences, 2021
27. Li S, Vaziri ND, Masuda Y, Hajighasemi-Ossareh M, Robles L, Le A, Vo K, Chan JY, Foster CE, Stamos MJ, Ichii H : Pharmacological activation of Nrf2 pathway improves pancreatic islet isolation and transplantation. Cell transplantation 24 : 2273-2283, 2015
28. Paupe V, Dassa EP, Goncalves S, Auchere F, Lonn M, Holmgren A, Rustin P : Impaired nuclear Nrf2 translocation undermines the oxidative stress response in Friedreich ataxia. PloS one 4 : e4253, 2009
29. Tsai TF, Chen PC, Lin YC, Chou KY, Chen HE, Ho CY, Lin JF, Hwang TI : Miconazole Contributes to NRF2 Activation by Noncanonical P62-KEAP1 Pathway in Bladder Cancer Cells. Drug design, development and therapy 14 : 1209-1218, 2020
30. Kaspar JW, Niture SK, Jaiswal AK : Nrf2:INrf2 (Keap1) signaling in oxidative stress. Free radical biology & medicine 47 : 1304-1309, 2009
31. Takasu C, Vaziri ND, Li S, Robles L, Vo K, Takasu M, Pham C, Farzaneh SH, Shimada M, Stamos MJ, Ichii H : Treatment with dimethyl fumarate ameliorates liver ischemia/reperfusion injury. World journal of gastroenterology 23 : 4508-4516, 2017
32. Takasu C, Vaziri ND, Li S, Robles L, Vo K, Takasu M, Pham C, Liu S, Farzaneh SH, Foster CE 3rd, Stamos MJ, Ichii H : Treatment With Dimethyl Fumarate Attenuates Calcineurin Inhibitor-induced Nephrotoxicity. Transplantation 99 : 1144-1150, 2015
33. Tonelli C, Chio IIC, Tuveson DA : Transcriptional Regulation by Nrf2. Antioxidants & redox signaling 29 : 1727-1745, 2018
34. Yuan Z, Zhang J, Huang Y, Zhang Y, Liu W, Wang G, Zhang Q, Yang Y, Li H, Chen G : NRF2 overexpression in mesenchymal stem cells induces stem-cell marker expression and enhances osteoblastic differentiation. Biochemical and biophysical research communications 491 : 228-235, 2017
35. Ling L, Nurcombe V, Cool SM : Wnt signaling controls the fate of mesenchymal stem cells. Gene 433 : 1-7, 2009
36. Hoepfner J, Kleinsorge M, Papp O, Ackermann M, Alfken S, Rinas U, Solodenko W, Kirschning A, Sgodda M, Cantz T : Biphasic modulation of Wnt signaling supports efficient foregut endoderm formation from human pluripotent stem cells. Cell biology international 40 : 534-548, 2016
37. Touboul T, Chen S, To CC, Mora-Castilla S, Sabatini K, Tukey RH, Laurent LC : Stage-specific regulation of the WNT/beta-catenin pathway enhances differentiation of hESCs into hepatocytes. Journal of hepatology 64 : 1315-1326, 2016
38. Jain AK, Jaiswal AK : GSK-3beta acts upstream of Fyn kinase in regulation of nuclear export and degradation of NF-E2 related factor 2. The Journal of biological chemistry 282 : 16502-16510, 2007
39. Teng Y, Zhao H, Gao L, Zhang W, Shull AY, Shay C : FGF19 Protects Hepatocellular Carcinoma Cells against Endoplasmic Reticulum Stress via Activation of FGFR4-GSK3beta-Nrf2 Signaling. Cancer research 77 : 6215-6225, 2017
40. Shams S, Mohsin S, Nasir GA, Khan M, Khan SN : Mesenchymal Stem Cells Pretreated with HGF and FGF4 Can Reduce Liver Fibrosis in Mice. Stem cells international 2015 : 747245, 2015