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Differential Expression of HNF4α Isoforms in Liver Stem Cells and Hepatocytes

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Abstract The hepatocyte nuclear factor (HNF4α) has been implicated in liver development and hepatocellular differentiation. The HNF4α expression in liver stem cells has not yet been fully investigated. Here, we describe the expression characterization of HNF4α in liver stem cells isolated from 2-acetylaminofluorene/partial hepatectomy (AAF/PH) rat model by a combination of density-gradient centrifugation and selective enzymatic digestion. The obtained cells were identified by flow cytometry (FCM) immediately and then by immunocytochemisty and reverse transcription-polymerase chain reaction (RT-PCR) after 5 d culture. The cultured stem cells were subjected to analysis of the expression of various HNF4α isoforms using RT-PCR, Western blotting, and electrophoretic mobility shift assay (EMSA). Results showed that HNF4α isoforms, α1, α2, α7, and α8 were present in liver stem cells, which contrasted with only α1 and α2 expression in hepatocytes. The distinct expression patterns of HNF4α isoforms in liver stem cells may contribute to maintain the ''stemness'' of these cells. J. Cell. Biochem. 99: 558–564, 2006.

Key words: hepatocyte nuclear factor 4α; isoform; liver stem cells; oval cells

Liver stem cells reside in the terminal bile ductules (canals of Hering) which are understood to constitute the stem cell niche in the adult liver [Paku et al., 2001]. These cells have been referred to as "oval cells" since Farber firstly described their shape in the rat liver [Farber, 1956]. When the regenerative capacity of hepatocytes is impaired in a rat with liver mass loss, oval cells can be activated to proliferate substantively [Novikoff et al., 1996]. Over the past decades, the phenotypic properties of the oval cells have been well defined. These cells are characterized by oval-shaped nucleus, small size and scant basophilic cytoplasms, and show features of both bile duct cells and fetal hepatocytes. They express alpha-fetoprotein (AFP), gamma-glutamyl transpeptidase (GGT), cytokeratin (CK) 7, CK8, CK18, CK19, etc. [Sirica et al., 1990]. Since cellular phenotype is the result of selective gene expression which is primarily controlled at transcriptional levels, knowledge of expression of transcription factors in oval cells is critical for understanding the characterization of oval cells.

Of numerous transcriptional factors in liver, hepatocyte nuclear factor (HNF4) has been considered to be at the top of the hierarchy of the transcription factor cascade that drives hepatocyte differentiation [Kuo et al., 1992]. HNF4α involves in the regulation of liver-specific gene expression and presents in cells with different isoforms depending on cellular differentiation extent [Torres-Padilla et al., 2001; Sugimoto et al., 2002]. HNF4α isoforms arise by either alternative usage of promoters or alternative RNA processing. Total nine HNF4α isoforms have been deduced in various species, but only four of them have been identified in rat [Sladek and Seidel, 2001]. It has been shown that HNF4α plays a central role in liver

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development and differentiation [Watt et al., 2003] and that HNF4α isoforms are involved in liver organogenesis by developmentally regulated styles [Torres-Padilla et al., 2001]. A number of investigations implied that adult liver stem cells originate from embryonic hepatoblasts and that the differentiation of adult liver stem cells recapitulates the organ development of liver [Alison et al., 2004]. Thus, identification of HNF4α isoforms in oval cells provides insight into transcriptional regulatory codes controlling a broad range of hepatic developmental programmes.

MATERIALS AND METHODS

Isolation and Culture of Oval Cells

Liver cell suspensions were prepared from Sprague–Dawley rats in the *N*-2-acetylaminofluorene/partial hepatectomy (AAF/PH) model [Golding et al., 1995] by a 2-step retrograde perfusion method [Aiken et al., 1990]. The suspension was centrifuged once at 50*g* for 2 min, and the cell pellet fraction was centrifuged again at 50*g* for 2 min. All of the supernatant fractions from each centrifugation were mixed and centrifuged at 200*g* for 6 min. Finally, the cell pellet, a nonparenchymal cell fraction, was resuspended in digestive medium consisting of DMEM/F12 plus 3% fetal bovine serum (FBS), 0.1% collagenase type IV, 0.1% Pronase E, and 0.004% DNase I and incubated twice each for 20 min at 37°C in a shaking water bath. After each incubation, the supernatant containing the released cells was decanted and filtered through a 60-μm mesh, and then cold DMEM/F12 with 3% FBS was added and centrifuged at 200*g* for 6 min at 4°C. The pellet was resuspended in Gey's Balanced Salt Solution and loaded onto the 50 of 70% Percoll and centrifuged at 800*g* for 30 min. After centrifugation, the cells banding at the interface between 50 and 70% Percoll were collected and washed twice. The final cell suspension was plated on culture ware at 1×10^4 cells/cm^2, and cultured at 37°C in a humidified incubator with 5% CO_2 in air. The medium consisted of DMEM containing 5% FBS, 10 ng/ml leukemia inhibitory factor (Sigma) and antibiotics, and was renewed every 2 or 3 days. Purified hepatocytes were obtained from untreated rats according to the reported method [Dunn et al., 1991] and used as controls in the following experiments.

Flow Cytometry (FCM)

Freshly isolated oval cells were fixed in 4% paraformaldehyde at 4°C for 10 min and washed with PBS containing 1% FBS. Nonspecific antigens were blocked by incubating the cells at room temperature for 1 h in permeabilization buffer (0.1% Saponin, 0.01 M HEPES, 1% FBS in PBS) containing 2% normal goat serum. After centrifugation at 200*g* for 5 min, the pellets were suspended in 0.5 ml permeabilization buffer and incubated with primary antibodies at 4°C for 30 min. The primary antibodies were OV-6 (an oval cell marker), AFP (gifts from Prof. Sell, Albany medical college, NY), and CK19 (Dako, Tokyo). The cells were washed in permeabilization buffer and then incubated in 100 μl permeabilization buffer containing FITC-conjugated goat anti-mouse IgG for 1 h. The cells were then washed and resuspended in 0.5 ml PBS at 5×10^4 cells/ml. The cells were assayed in a flow cytometer (Becton Dickinson, San Jose, CA), and the data were analyzed with Cell-Quest software. For an isotype control, nonspecific mouse IgG was substituted for the primary antibody.

Immunofluorescence Staining

Oval cells grown on glass coverslips were fixed in cold 2% paraformaldehyde containing 0.1% Triton X-100 for 30 min. After a rinse in PBS, nonspecific binding was blocked with 10% normal goat serum. The cells were stained with antibodies directed against OV-6, AFP, and CK19 for 1 h at 37°C. After rinsing with PBS, the cells were incubated for 30 min with FITC-labeled secondary antibodies. After rinsing with PBS, the cells were observed with a fluorescent microscope (Olympus, Japan). All staining procedures were performed at room temperature. All antibodies were diluted in 10% skim milk/PBS.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNA was prepared from the cultured oval cells and fresh hepatocytes by using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. RNA amounts were quantified by measuring absorbance at 260 nm. RT was performed using SuperScript II Moloney murine leukemia virus reverse transcriptase and oligo(dT)18 primers on 5 μg of DNase I-treated RNA. Aliquots of cDNA were

TABLE I. PCR Primers

Gene name	Primer (forward)	Primer (reverse)	Annealing temperature (°)	Product size (bp)	GenBank accession number
CK7	tctcgctccactgcctatc	ggctgctcttggctgact	58	370	XM_217035
CK8	atgaaccgtaacatcagcc	ttcaatcttcttcacgacca	56	474	AY464139
CK18	tcttgccgctgatgactt	tttgggagcatccacttc	53	250	X81448
CK19	tcagacctgcgtcccttat	acctccagctcgccatta	58	384	AY464140
GGT	ttccaacccagcatccaa	ggcagaacacctcgcaca	54	121	X03518
TAT	atccctgtggctccgtgtt	cctgctccgcaatcaacc	58	546	M18340
HNF4α1/α2	ccttggacccagcctaca	gcttgaggctccgtagtgt	58	175	NM_022180
HNF4α7/α8	tccatgctccccagtgtc	gcttgaggctccgtagtgt	58	199	AF329936
HNF4α1/α7	gggcaccaatgtcatagt	aggagcttgtaggattcag	52	123	NM_022180
HNF4α2/α8	gggcaccaatgtcatagt	aggagcttgtaggattcag	52	153	NM_022180

subsequently amplified using specific primers for CK7, CK8, CK18, CK19, GGT, TAT (tyrosine aminotransferase), and HNF4α isoforms listed in Table I. The PCR cycles consisted of denaturation at 94°C for 30 s, annealing for 45 s, and extension at 72°C for 30 s, for 35 cycles. Amplified PCR products were analyzed by 1% agarose gel or 10% polyacrylamide gel electrophoresis (PAGE) and ethidium bromide staining.

Nuclear Extracts

Nuclear extracts were prepared from cultured oval cells and fresh hepatocytes according to the manufacturer's recommendations. Briefly, the protease inhibitor cocktail (Sigma, P8340) was diluted at 1:100 into Pierce's nuclear and cytoplasmic extraction reagents (Pierce, 78833). The cytoplasmic extraction reagent was used to remove cell membranes and cytoplasmic contents. The left nuclei were treated with the nuclear extraction reagent. The supernatants (nuclear extracts) were measured by BioRad protein assay reagents using bovine serum albumin as standard. The nuclear extracts were stored in −20°C after boiling with Western Blotting loading buffer for 5 min or frozen in liquid nitrogen after dialysis in Micro DispoDialyzer (Sigma, D9062) for 2 h. The dialysis buffer consisted of 20 mM HEPES, pH 7.9, 100 mM KCl, 0.2 mM EDTA, 20% glycerol, 1 mM DTT, and 0.5 mM PMSF.

Western Blotting

Sixty micrograms of nuclear proteins were separated by SDS–12.5% PAGE followed by transfer to nitrocellulose membrane. The membrane was preincubated in blocking buffer (5% nonfat dried milk in PBS) and then incubated for overnight at 4°C with a 1:2,500 dilution of α445 antiserum (Sladek Lab, University of California, Riverside, CA) in blocking buffer. The membrane was washed in washing buffer (PBS containing 0.05% Tween-20) and incubated for 2 h at room temperature with a 1:5,000 dilution of goat anti-rabbit IgG conjugated to horseradish peroxidase (Dako). The blot was washed three times in washing buffer and developed with SuperSignal West Pico Chemiluminescent Substrate (Pierce, 34077).

Electrophoretic Mobility Shift Assay (EMSA)

Nuclear extracts were incubated at room temperature with ^{32}P-labeled oligonucleotides containing HNF4α-binding site of the human apo CIII promoter (5′-*g*agcaggtgacctttgcccagcgc-3′, 5′-*ga*gcgctgggcaaaggtcacctgc-3′) in 10 μl of Gelshift-binding buffer (Promega). For competition assays, nuclear extracts were preincubated with a 100-fold molar excess of unlabeled probe for 30 min before the addition of labeled probe. For supershift assays, αN1.14 (Sladek Lab) or α445 antiserum was added after 15 min, and the incubations were continued for another 20 min before they were loaded onto a 6% native polyacrylamide gel in 0.5 × TBE. The gel was preelectrophoresed at 200 V at 4°C for approximately 40 min, loaded, and run again for 2 h. After drying, the gel was subjected to autoradiography for 3 days at −20°C.

RESULTS

Identification of Oval Cells

Oval cells were isolated from rat liver in the AAF/PH model and propagated in vitro. To obtain as many oval cells as possible, the isolation was performed at the ninth day after PH when the amount of oval cells attained at peak and remained undifferentiated characteristics [Nagy et al., 1994]. We isolated the oval cells with a combination of density-gradient

centrifuge and selective enzymatic digestive method. The yield of oval cells was about 10^7 cells per liver. The freshly isolated oval cells displayed typical morphological characteristics, namely small cell size, oval nuclei, and a high nucleus to cytoplasm ratio. To determine whether the above procedures allowed for the enrichment of oval cells, we performed flow cytometry (FCM) analysis by using the antibodies against OV-6, AFP, and CK19. As shown in Figure 1A, the percentage of freshly isolated oval cells positive for OV-6, AFP, or CK19 was between 80 and 90%. Hepatocytes were negative for these markers (data not shown).

For the purpose of eliminating the contamination of parenchymal cells and expanding the population of oval cells, we cultured the oval cells in vitro for 5 d. The method was proven to be effective. The procedure for the isolation of oval cells was harmful to hepatocytes, as indicated by hepatocellular loss of attachment to culture ware. The floating damaged hepatocytes could be removed from the cultures by medium exchange. Elimination of hepatocytes from the oval cells was confirmed by their lack of the transcripts of TAT, a specific marker for mature hepatocytes (Fig. 1C). Adherent oval cells proliferated into colonies, which consisted of decades of cells after 5 d. The colonies were positive for OV-6, AFP, and CK19 continuously (Fig. 1B) and expressed biochemical markers specific for biliary cells (CK7/19, GGT) and immature hepatocytes (CK8/18) (Fig. 1C), indicating that the cultured oval cells retained the phenotypic features of bipotential stem

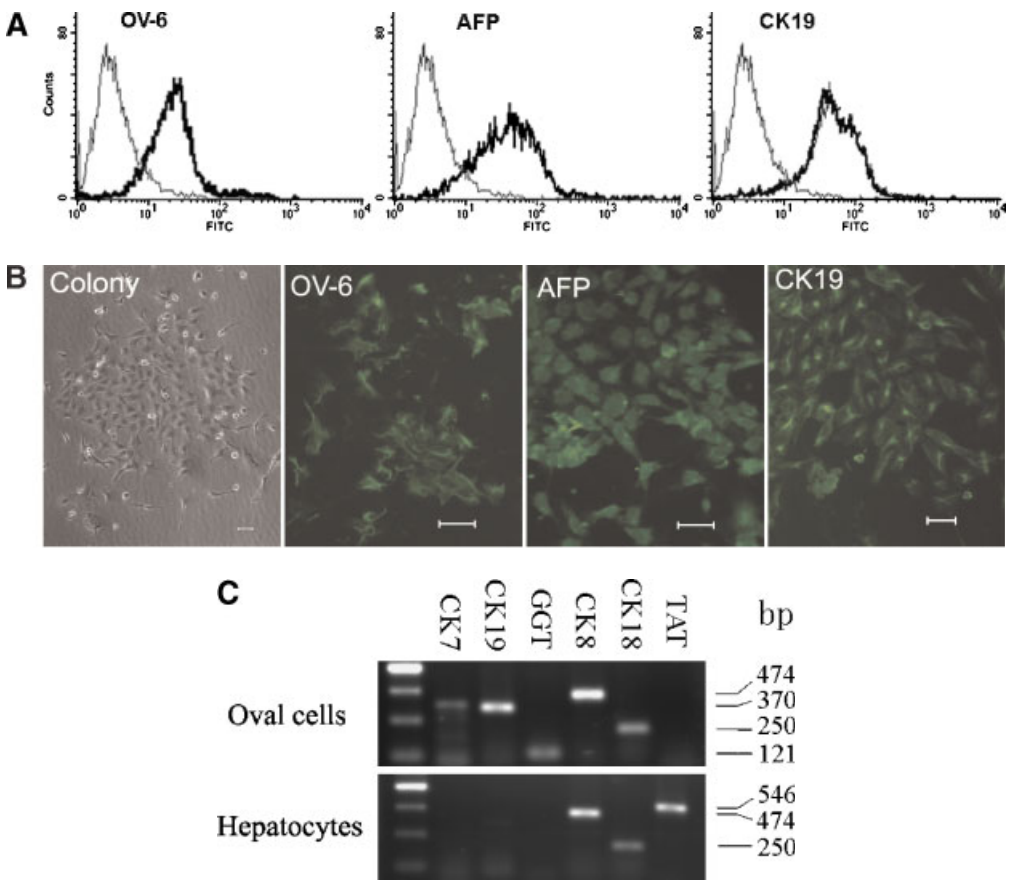

Fig. 1. Identification of oval cells through discern of the given markers. **A**: Flow cytometric analysis for phenotypic markers of freshly isolated oval cells. Isolated oval cells were labeled with primary antibodies against OV-6, AFP, CK19 (thick lines), or IgG isotype controls (thin lines) and then with FITC-conjugated secondary antibodies. **B**: Immunofluorescency of cultured oval cells. Single oval cell attached on a culture dish and formed a relatively large colony after 5 d of culture. The cell colonies were identified by staining for OV-6, AFP, and CK19. Scale bar, 20 μm. **C**: Agarose gel electrophoresis of RT-PCR analysis for phenotypic markers. Total RNA was prepared from oval cells cultured for 5 d, and fresh hepatocytes served as controls. Experiments were done three times, with similar patterns. Results from typical experiments are shown. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

cells. The results support the idea that the short-term cultivation can be used to purify and expand oval cells without loss of stem cell characterization.

Characterization of HNF4α in Oval Cells

To characterize the expression patterns of HNF4α isoforms in oval cells, their transcripts, proteins, and DNA-binding activity were detected. The locations of the primers for reverse transcription-polymerase chain reaction (RT-PCR) and recognition sites of antibodies were indicated in Figure 2A. The overall expression patterns of HNF4α isoforms in the cultured oval cells were distinctly different from those observed in freshly isolated hepatocytes. RT-PCR analysis revealed that the investigated four mRNA isoforms of HNF4α were all present in the cultured oval cells. This was contrasted to the hepatocytes which lacked expression of HNF4α7/α8 (Fig. 2B). For further verification in protein level, Western blotting analysis was performed with α445 antibody. The antibody generated using synthesized peptide as an immunizing agent, was able to recognize the shared C-terminal region of HNF4α1, 2, 7, and 8. As shown in Figure 2C, four bands appeared in the lane uploaded with oval cell lysates, while only two bands existed in the lane to which hepatocyte lysates were loaded on. We investigated the

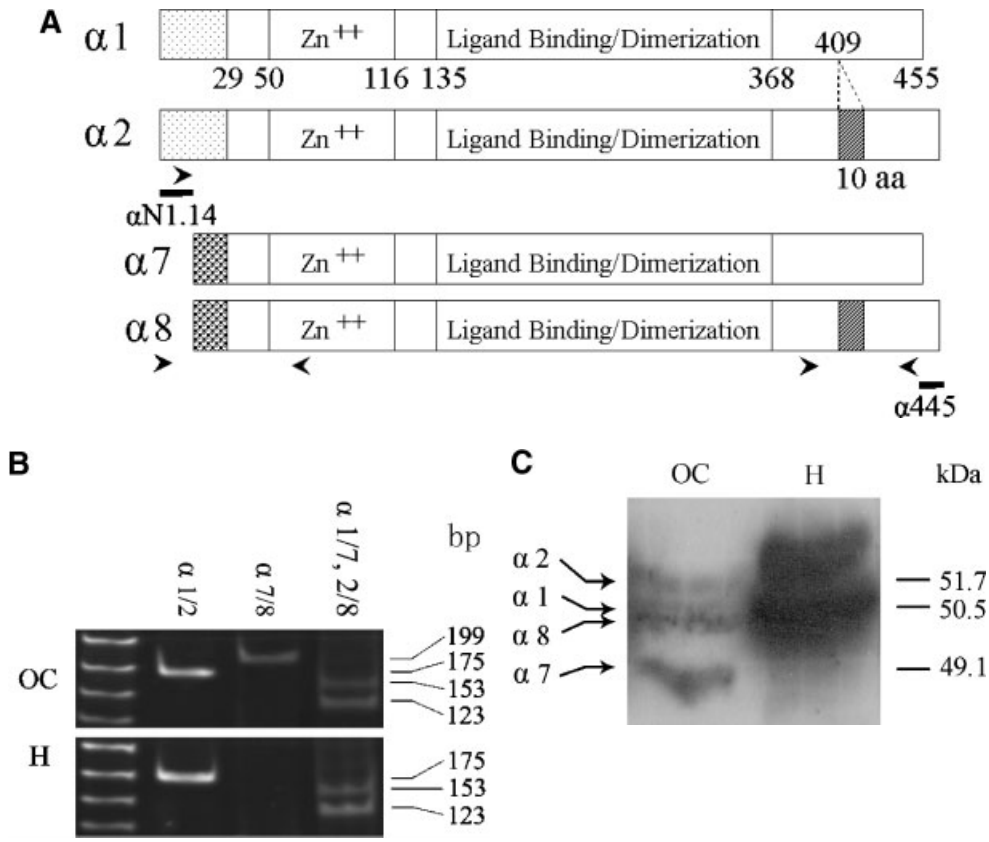

Fig. 2. Expression of HNF4α isoforms in oval cells and hepatocytes. **A**: Diagram of HNF4α isoforms (modified from the figure on http://www.sladeklab.ucr.edu/info.html). Different N- and C-terminal structures among HNF4α isoforms are indicated. Arrowheads indicate positions of PCR primers and bars recognition sites of α445 and αN1.14 antiserum. **B**: PAGE electrophoresis of RT-PCR analysis for HNF4α isoform expression. PCR primers were common to some isoforms as described in Table I. **C**: Western blotting analysis of equivalent amounts of cellular nuclear extracts. The corresponding positions of each HNF4α isoform are indicated. **D**: EMSA of cellular nuclear extracts. Presence of nuclear extracts and competitor as well as antibodies are as indicated on top. The positions of the shifted HNF4α DNA-binding complexes and the supershifted immune complexes are indicated. The cells used for mRNA and nuclear protein extraction were oval cells cultured for 5 d or freshly isolated hepatocytes. OC, oval cells; H, hepatocytes. Experiments were done three times, with similar patterns. Results from typical experiments are shown.

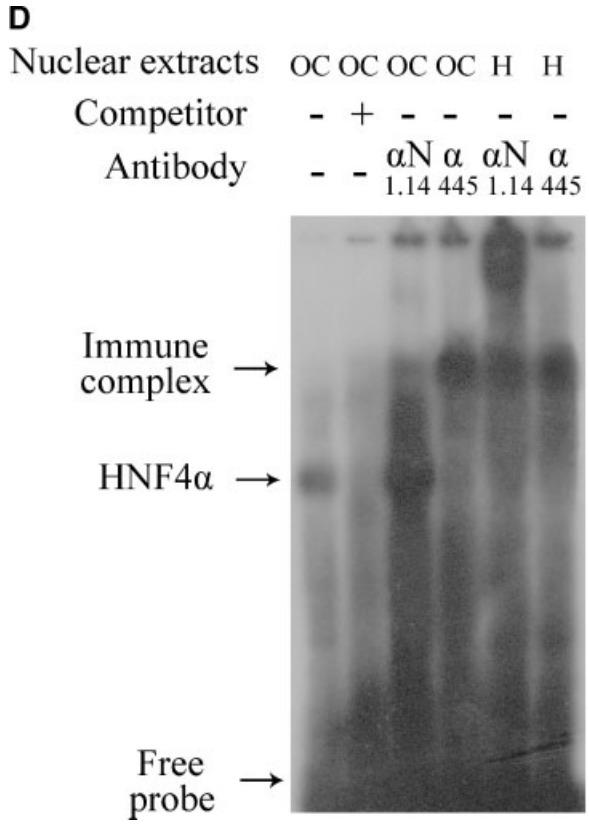

Fig. 2. (*Continued*)

respective DNA-binding pattern of HNF4α in nuclear extracts prepared from oval cells and hepatocytes. The HNF4α isoforms binding to human apo CIII promoter were identified using αN1.14 and α445 antisera, respectively. The αN1.14 antibody recognized the common N-terminus of HNF4α1/α2. The complex formed with oval cell nuclear extracts was completely supershifted by the α445 antibody, but not by the αN1.14 antibody (Fig. 2D, lanes 3 and 4). The complex could not be displaced by increasing amounts of αN1.14. Conversely, the complexes formed with hepatocyte nuclear extracts were completely supershifted by both αN1.14 and α445 antibodies (Fig. 2D, lanes 5 and 6). That indicated HNF4α from both kinds of cells reacted with α445 antibodies completely, but only a fraction of HNF4α in oval cells reacted with αN1.14 antibody.

DISCUSSION

In the study of Nakhei et al. [1998], they found trace amounts of HNF4α7 transcripts initiated from P2 promoter in normal liver and speculated that the transcripts might be present in a stem cell compartment. However, the speculation has never been testified in oval cells. To address this question, the pure and sufficient oval cells have to be prepared and identified first. Under physiological conditions, hepatic stem cells account for a little amount of cell population in an adult liver. But the stem cells could be activated and proliferated following dosing animals with toxins or carcinogens, alone or combined with other surgical or dietary regimens [Grompe and Finegold, 2001]. AAF/PH protocol is one of the widely used models, of which oval cells emerge prolifically as the activated progeny of a dormant stem cell compartment in the liver. In this way, the oval cells can be isolated with satisfactory greater than 90% purity [Fausto et al., 1987].

In the work reported here, the isolated oval cells displayed highly purity indicated by FCM analysis. Due to the lack of hallmarker of oval cells, it is hard to determine accurately the purity of oval cells. We employed combined biochemical features to determine the purity of the freshly isolated oval cells. The instant oval cells expressed OV-6, AFP, and CK19, which represent the markers of oval cells, immature hepatocytes, and biliary epithelial cells, respectively. The short cultivation procedure not only propagated the oval cells but also helped to purify them. This was demonstrated by the colony growth of oval cells and the removal of contaminated hepatocytes. The oval cells cultured for 5 d retained the characterization of bipotential stem cell indicated by expression of cholangiocyte lineage markers CK7/19, GGT and immature hepatocyte lineage markers CK8/18. In the cultured oval cells, all of the HNF4α isoforms were present at mRNA and protein levels. This finding supports the hypothesis that products of HNF4α P2 promoter are restricted to a subpopulation of the stem cell compartment in the liver [Nakhei et al., 1998]. In addition, this study suggests that oval cells possess the HNF4α expression patterns of both fetal and adult hepatic phenotypes [Torres-Padilla et al., 2001].

The significance of the different HNF4α isoforms in oval cells has been not yet known. HNF4α7 was identified first as an embryonic isoform and thought to be relevant for the acquisition and maintenance of an embryonic/fetal phenotype in hepatocytes. The transcript and its splicing variant HNF4α8 fade away

along with embryonic hepatoblast differentiation [Nakhei et al., 1998; Torres-Padilla et al., 2001]. HNF4α7/8 isoforms exhibit weaker transcriptional potentials than HNF4α1/2 ones [Eeckhoute et al., 2003]. Different HNF4α isoforms heterodimerize with one another and have different ability to recruit co-factors [Torres-Padilla et al., 2001, 2002]. Therefore, the expression of all HNF4α isoforms in oval cells may contribute to the establishment of an appropriate HNF4α transcriptional activity and regulative connections that play critical roles in the maintenance of oval cell features. It may be inferred that the transcriptional network controlled by the HNF4α isoforms is indispensable for the maintenance of developmental programmes prestored in oval cells.

REFERENCES

Aiken J, Cima L, Schloo B, Mooney D, Johnson L, Langer R, Vacanti JP. 1990. Studies in rat liver perfusion for optimal harvest of hepatocytes. J Pediatr Surg 25:140–144.

Alison MR, Vig P, Russo F, Bigger BW, Amofah E, Themis M, Forbes S. 2004. Hepatic stem cells: From inside and outside the liver? Cell Prolif 37:1–21.

Dunn JC, Tompkins RG, Yarmush ML. 1991. Long-term in vitro function of adult hepatocytes in a collagen sandwich configuration. Biotechnol Prog 7:237–245.

Eeckhoute J, Moerman E, Bouckenooghe T, Lukoviak B, Pattou F, Formstecher P, Kerr-Conte J, Vandewalle B, Laine B. 2003. Hepatocyte nuclear factor 4 alpha isoforms originated from the P1 promoter are expressed in human pancreatic beta-cells and exhibit stronger transcriptional potentials than P2 promoter-driven isoforms. Endocrinology 144:1686–1694.

Farber E. 1956. Similarities in the sequence of early histological changes induced in the liver of the rat by ethionine, 2-acetylamino-fluorene, and 3′-methyl-4-dimethylaminoazobenzene. Cancer Res 16:142–148.

Fausto N, Thompson NL, Braun L. 1987. Purification and culture of oval cells from rat liver. In: Pretlow TG II, Pretlow TP, editors. Cell separation—Methods and selected applications. Orlando: Academic Press. pp 45–77.

Golding M, Sarraf CE, Lalani EN, Anilkumar TV, Edwards RJ, Nagy P, Thorgeirsson SS, Alison MR. 1995. Oval cell differentiation into hepatocytes in the acetylaminofluorene-treated regenerating rat liver. Hepatology 22:1243–1253.

Grompe M, Finegold MJ. 2001. Liver stem cells. In: Marshak DR, Gardner RL, Gottlieb D, editors. Stem cell biology. New York: Cold Spring Harbor Laboratory Press. pp 455–497.

Kuo CJ, Conley PB, Chen L, Sladek FM Jr, Darnell JE, Crabtree GR. 1992. A transcriptional hierarchy involved in mammalian cell-type specification. Nature 355:457–461.

Nagy P, Bisgaard HC, Thorgeirsson SS. 1994. Expression of hepatic transcription factors during liver development and oval cell differentiation. J Cell Biol 126:223–33.

Nakhei H, Lingott A, Lemm I, Ryffel GU. 1998. An alternative splice variant of the tissue specific transcription factor HNF4α predominates in undifferentiated murine cell types. Nucleic Acids Res 26:497–504.

Novikoff PM, Yam A, Oikawa I. 1996. Blast-like cell compartment in carcinogen-induced proliferating bile ductules. Am J Pathol 148:1473–1492.

Paku S, Schnur J, Nagy P, Thorgeirsson SS. 2001. Origin and structural evolution of the early proliferating oval cells in rat liver. Am J Pathol 158: 1313–1323.

Sirica AE, Mathias GA, Sano N, Elmore LW. 1990. Isolation, culture and transplantation of intra-hepatic biliary epithelial cells and oval cells. Pathobiology 58: 44–64.

Sladek FM, Seidel SD. 2001. Hepatocyte nuclear factor 4α. In: Burris TP, McCabe ERB, editors. Nuclear receptors and genetic disease. San Francisco: Academic Press. pp 309–361.

Sugimoto S, Mitaka T, Ikeda S, Harada K, Ikai I, Yamaoka Y, Mochizuki Y. 2002. Morphological changes induced by extracellular matrix are correlated with maturation of rat small hepatocytes. J Cell Biochem 87:16–28.

Torres-Padilla ME, Fougere-Deschatrette C, Weiss MC. 2001. Expression of HNF4α isoforms in mouse liver development is regulated by sequential promoter usage and constitutive 3′ end splicing. Mech Dev 109:183–193.

Torres-Padilla ME, Sladek FM, Weiss MC. 2002. Developmentally regulated N-terminal variants of the nuclear receptor hepatocyte nuclear factor 4alpha mediate multiple interactions through coactivator and corepressor-histone deacetylase complexes. J Biol Chem 277:44677–44687.

Watt AJ, Garrison WD, Duncan SA. 2003. HNF4: A central regulator of hepatocyte differentiation and function. Hepatology 37:1249–1253.