[0028] Figures 5A-5I illustrate a variety of CYP 450 enzyme activities in the differentiated hepatocyte-like cells. Phase I-II CYP450 enzyme activity is estimated by drug-drug interaction and detoxification tests (inducer, inhibitor), including CYP1A2 (Figure 5A), CYP2B6 (Figure 5B), CYP2C8 (Figure 5C), CYP2C9 (Figure 5D), CYP2C19 (Figure 5E), CYP2D6 (Figure 5F), CYP2E1 (Figure 5G), CYP3A4 (Figure 5H), and CYP 7A1 (Figure 5I). Inducer and inhibitor used as (rifampin, Rif and ciprofloxacin, Cip), (phenobarbital, phen and Cip), (Rif and gemfibrozil, Gem), (Rif and Gem), (Rif and ticlopidine,Tico), (Rif as inducer only), (Rif as inducer only), (Rif, and itraconazole, Itra), and (THA, 2,4,6-trihydroxyacetophenone and CDCA, chenodeoxycholic acid), respectively. hTS indicating hTS cells, hTHL indicating human trophoblast-derived hepatocyte-like cells; and Huh7 indicating human hepatoma Huh7 cells.

[0029] Figure 6 illustrates a table showing biomarker expression (e.g. mRNAs) during the different stages of a hepatocyte differentiation.

[0030] Figure 7 illustrates the cellular processes of DE formation by TissueFAX analysis. bFGF (10 ng/mL) was used to induce differentiation of hTS cells to mesendoderm indicated by upregulation of MIXL1 (black). Subsequently, the mesendoderm differentiated into DE lineage at about 4 hours induction, expressing a downregulation of MIXL1 (grey). n indicates the total number of cells counted.

[0031] Figure 8 illustrates expression level analysis of biomarkers described herein. Figure 8A illustrates that FGFR inhibitor (PD166866) blocks bFGF-induced PI3K with β-actin as a loading control. Figure 8B illustrates that PI3K siRNA inhibits the expression of PI3K and p-AKT. Cells transfected with non-specific shRNA were used as control. β-actin was used as a loading control. Figure 8C shows that siRNAs against AKT subunits inhibits the bFGF-induced expression of p-AKT and p-CREB1. Cells transfected with non-specific shRNA were used as control. β-actin was used as a loading control. Figure 8D shows that AKT interacts directly to CREB1 by IP assay.

[0032] Figures 9A to 9F illustrate the genetic fluctuation profiles of hepatic development-associated 31 genes after induction by qPCR analysis in hTS cells, the genes shown in Figures 9A to 9F.

[0033] Figures 10A-10D and 10AS-10FS illustrate immunoreactive markers during DE formation. (10A) Western blot analysis in time course of representative markers of primitive streak and DE markers at the initial induction (8 hr). Data indicating mean ± SD, n = 3, *: $p < 0.05$ as statistic significant. (10B) immunocytochemistry of Foxa2 and Sox17 (left panel), Gsc (middle panel), and Mixl1 (right panel) at 4 hr of bFGF induction. (10C) Identification of CREB1 in targeting at three sites of promoter (upper panel) in miR-124a to increase its levels by ChIP-qPCR. C: as control. Data representing mean ± SD, n = 3, *: $p < 0.05$ as statistic significant. (10D) bFGF induces a parallel

expression between phosph(p)-CREB1 and miRNA-124a by qPCR assay. Data indicating mean ± SD, n = 4, *: $p < 0.05$ as statistic significant. (10AS) A shifting mean Mixl1 intensity in cells within 4 hr induction by TissueFAX analysis. Blank area as control, blue area as mesendoderm stage, red area as DE stage. (10BS). FGFR inhibitor (PD 166866) blocks the bFGF-induced PI3K by Western blotting. β-actin was used as loading control. (10CS) PI3K siRNA inhibits expression of PI3K and p-Akt. Cells transfected with non-specific shRNA are used as control. β-actin was used as loading control. (10DS) siRNAs against Akt subunits inhibits the bFGF-induced expressions of p-Akt and p-CREB1. Cells transfected with non-specific shRNA are used as control. β-actin was used as loading control. (10ES) Akt interacts directly to CREB1 by IP assay. (10FS) bFGF-induced miR-124a is inhibited by using CREB1 shRNAs. Data representing mean ± SD, n = 3, *: $p < 0.05$ as statistic significant.

[0034] Figures 11A-11I and 11AS-11CS illustrate molecular mechanisms for DE specification. (11A, 11B, 11C) Luciferase reporter assays of miR-124a repressing the expressions of Smad4 plasmid (pSmad4) (A), pGSK3β (B), and pCdx2 (C) via targeting the promoter(s) of gene (upper panel). Empty vector: control, Data indicating mean ± SD, n = 3, *: $p < 0.05$ as statistic significant. (11D) β-catenin binds to the region (-2.1 kb) of promoter in *Foxa2* gene over time by ChIP-qPCR assay. Error bars indicate SD of 3 replicates. (11E) Foxa2 targets the promoter of *Betatrophin* by ChIP assay, showing production of betatrophin at 12 hr induction (arrow). Input: whole cells as positive control. IgG as negative control. (11F) Expression of various transcription factors in response to miR-124a and anti-miR-124a antibody at 4 hr of bFGF induction by Western blot analysis. β-actin: loading control. (11G) A reciprocal inhibitory function between Cdx2 (green) and Oct4 (red) at 4 hr of bFGF induction immunocytochemically. (11H) Oct4 binds to two regions (-1 and -1.8 kb) of promoter in *Sox17* gene at 2 hr of bFGF induction by ChIP-qPCR assay. Error bars indicate SD of 3 replicates. (11I) Schematic illustration of molecular regulation in DE differentiation of hTS cells. (11AS) Smad4 shRNAs inhibit expression of Mixl1 by immunoblotting assay. (11BS) Expression of Oct4, Cdx2, Nanog, and Sox2 in time course during DE formation. Data indicating mean ± SD, n =3, *: $p < 0.05$. (11CS) Immunoreactive Albumin (ABL) and AFP express in the hepatocyte-like cells.

[0035] Figures 12A- 12D and 12AS -12BS illustrate biological characteristics of hepatocyte-like cells. (12A) H&E staining of the crescent cell mass (left panel, insert). Numerous clustered cells distributed irregularly at the outer peripheral layer containing abundant small embryonic progenitor-like cells with condensed nuclei (right lower). Columnar tree-like ECMs along with hepatocyte-like cell linings radiating from the peripheral layer to the central areas (right upper). (12B) Hepatocyte-like cells in the CCl4-damaged liver tissues, showing immunoreactive stem-121-positive cells (left upper), C-*kit* positive cells (right upper, arrow), CK19 (left lower, arrow), and CK18 (right lower,

arrow). (12C) A variety of specific immunoreactive markers during liver development observed in the crescent cell mass histologically. Cellular surface markers make up polygonal shape of hepatocyte seen in small insert. (12D) Representative electron micrographs showing: a large cytoplasm/nucleus ratio, plenty of mitochondria (m), endoplasmic reticulum (rer), lipid droplets, space of Disse (SD), extracellular matrix (ecm), nucleus (n), and sinusoid in upper micrograph, glycogen (gly) storage with rosette formation (red circle) in lower left micrograph, and lower right micrographs showing bile canaliculus lumen (bc) and junctional complex (upper) and tight junction (lower). (12AS) Immunoreactive albumin (ALB) and AFP expressed in the hepatocyte-like cells as shown in the figure. (12BS) Morphological changes during hTS cells differentiation to hepatocyte-like cells in time course.

[0036] Figures 13A-13B and 13AS illustrate secretomics in hepatocyte-like cell culture medium. (13A) Proteomic analysis of culture medium before cell culture (as control, left panel) and after 5-day cell culture (right panel) revealing a new formation of protein (designated as No. 413, circle). (13B) No immunoreactive TGFβ1, collagen IV (COL4), and fibronectin (FN) expressed in hTS cells before induction (upper panels) and after induction for 5-days, coexpression of them distribute as columnar ECMs between hepatocyte-like cells in 3-D structure (lower panels). (13AS) Mascot MS/MS ions search system analysis 20151001_LiP_413, transforming growth factor-beta-induced protein ig-h3 precursor [Homo sapiens].

[0037] Figures 14A-14E illustrate functional Characteristics of Hepatocyte-Like Cells. (14A) HepatoHepatocyte-like cells secrete albumin (left) and urea (right; by stimulation of 5 mM ammonium chloride for 1-day) into the cultured medium by automatic analyzer (Hitachi 7080; Tokyo, Japan). Error bars indicate SD of 3 replicates. **: $p < 0.01$. (14B) LDL uptake assay shows immunoreactive LDL (red, left), LDL receptor (LDLR, green, middle), and their emerged image (right) in the cells. (14C) Oil-O-Red test shows fat droplets (red) in the cells. (14D) Glycogen storage test identifies the presence of glycogen (pink and red) by periodic acid–Schiff (PAS) staining using diastase treatment (upper panel) and also by fluorescent PAS staining (lower panel). (14E) A variety of phase I-II CYP 450 enzyme activity in response to inducer (green) and inhibitor (pink) at 24 hr treatment in hepatocyte-like cells by qPCR analysis. Abbreviations: Rif, rifampin; Cip, ciprofloxacin; Itra, itraconazole; Phen, phenobarbital; Gem, gemfibrozil; THA, 2,4,6-trihydroxyacetophenone; CDCA, chenodeoxycholic acid; and Tico, ticlopidine.

[0038] Figures 15A-15F illustrate responsiveness of intravenous transplantation by hepatocyte-like cells (15A) Serum levels of AST and ALT are higher in cell therapy group (CCl4 + cells; n = 8) than control group (CCl_4 only; n = 8) over time. Data represent mean ± s.e.m., n =