HEPATOCYTE NUCLEAR FACTOR (HNF)-4α INDUCES TRANSDIFFERENTIATION OF HEMATOPOIETIC CELLS INTO HEPATOCYTES

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Hematopoietic stem cells can directly transdifferentiate into hepatocytes due to cellular plasticity, but the molecular basis of transdifferentiation is not known. Here we show the molecular basis using lineage-depleted oncostatin M receptor β-expressing (Lin−OSMRβ+) mouse bone marrow cells in a hepatic differentiation culture system. Differentiation of the cells was marked by the expression of albumin. Hepatocyte nuclear factor (HNF)-4α was expressed and translocated into the nuclei of the differentiating cells. Suppression of its activation in OSM-neutralized culture medium inhibited cellular differentiation. Ectopic expression of full-length HNF4α in 32D myeloid cells resulted in decreased myeloid colony-forming potential and increased expression of hepatocyte-specific genes and proteins. Nevertheless, the neo-hepatocytes produced in culture expressed active P450 enzyme. The obligatory role of HNF4α in hepatic differentiation was confirmed by transfecting Lin−OSMRβ+ cells with dominant-negative HNF4α in the differentiation culture because its expression inhibited the transcription of the albumin and tyrosine aminotransferase genes. The loss and gain of functional activities strongly suggested that HNF4α plays a central role in the transdifferentiation process. For the first time, this report demonstrates the mechanism of transdifferentiation of hematopoietic cells into hepatocytes, in which HNF4α serves as a molecular switch. Most adult organs and tissues possess tissue-specific adult stem/progenitor cells. Adult stem cells regenerate damaged tissue to maintain normal tissue homeostasis following trauma, disease or aging (1). In spite of the presence of tissue-specific adult stem cells, numerous reports have suggested that some organs recruit stem cells from other sources (2,3). Bone marrow (BM)-derived stem cells have been found to be involved in the regeneration of damaged skeletal muscle, neurons, hepatocytes and other tissues (4-7). Regeneration of non-hematopoietic tissue by hematopoietic cells has been proposed as a promising novel class of therapies for the treatment of many diseases and injuries in mammals. The changes in phenotype and functional properties of somatic stem cells into a completely different type of cell have been termed transdifferentiation (8). The cause of transdifferentiation is thought to be changes in the expression of a master gene(s), which encodes a transcription factor(s) that directs the differentiation program for the cellular fate (9). The reprogramming of cells may also be influenced by local microenvironmental cues. The molecular basis of transdifferentiation has been investigated using different in vitro models. The first report described the derivation of muscle cells by induced expression of a muscle-specific transcription factor (MyoD) in fibroblasts, pigment and nerve cells (10). Later, it was reported that ectopic expression of the adipogenic transcription factors PPARγ and C/EBPα in murine G8 myoblasts induced differentiation into mature adipocytes (11).
The differentiated cells not only expressed molecular markers of adipogenic lineage but the cellular reprogramming also resulted in the suppression of myogenic transcription factors (11). Besides ectopic expression of adipogenic transcription factors, disruption of the Wnt-signaling pathway also caused adipogenic differentiation in myoblasts (12), suggesting that the local microenvironment can induce reprogramming of cells. The molecular basis for differentiation of pancreatic to hepatic cells has also been studied. It has been shown in a pancreatic cell line that the transcription factor C/EBPβ (CCAAT enhancer binding protein) acts as a molecular switch for differentiation (13).

Many investigators have shown that mouse and human hematopoietic stem cells can transdifferentiate into hepatocytes in different mouse models (14-18). In vitro studies also demonstrated the hepatic differentiation potential of mouse and human hematopoietic cells (19-23). Hepatocyte nuclear factor (HNF)4α, a transcription factor of the nuclear hormone receptor family, is expressed in the hepatic diverticulum at the onset of liver development (24,25). It is essential and plays a central role in liver gene expression and hepatic differentiation. Mouse embryos lacking HNF4α do not complete the gastrulation process due to failure to express metabolic proteins, serum factors and several apolipoproteins (26). However, none of the earlier studies (14-23) described the molecular basis of transdifferentiation. Previously, we have described an in vitro culture system for transdifferentiation of Lin-OSMRβ+ BM cells into hepatocytes (18,23). In this study, we report that HNF4α acts as a molecular switch for transdifferentiation of BM-derived cells into hepatocytes.

EXPERIMENTAL PROCEDURES

Cells and culture medium- Lin-OSMRβ+ cells were purified from 8-week-old FVB/NJ mice bone marrow cells (BMC) by a two-step magnetic-activated cell sorting technique (Supplemental Methods). The purified cells were cultured in LabTek chamber slides as well as in 6-well plates at a cell density of 5 x 10^4 cells/cm² in the presence of Iscove’s modified Dulbecco’s medium (IMDM) supplemented with 10% damaged liver serum (DLS) and 1 μM dexamethasone (23). The DLS was collected from the mice 24 h after intraperitoneal injection of a 10% CCl₄ solution (200 μl) in mineral oil (18). Murine myeloid cells (32Dcl3) were cultured in RPMI-1640 supplemented with 10% fetal calf serum and 5 ng/ml interleukin-3 (Papertotech Asia, Israel). 32Dcl3 is a hematopoietic cell line that can terminally differentiate into neutrophils in the presence of G-CSF (27). Experiments using mouse primary cells were conducted according to procedures approved by the Institutional Animal Ethics Committee.

Bio-neutralization of OSM- Prior to initiation of the culture, the medium was incubated with anti-OSM antibody (5 μg/ml) at 37°C for 2 h. To maintain the neutralization, the medium was supplemented with fresh antibody. The OSM-neutralizing antibody was purchased from R&D Systems (Minneapolis, MN).

Plasmids and transient transfection- 32Dcl3 and Lin OSMRβ+ cells were transfected with the full-length (FL) and dominant-negative (DN) HNF4α constructs, respectively. The FL- and DN-HNF4α genes were cloned into the pcDNA3.1 vector under the control of the CMV promoter (28,29). DN-HNF4α is a selective dominant-negative mutant, which forms defective heterodimers with wild-type HNF4α, thereby preventing DNA binding and subsequent transcriptional activation by HNF4α (29). 32Dcl3 cells (1 x 10^5/well) were transfected with 1 μg of the FL-HNF4α construct using the DreamFect reagent (OZ Biosciences, Marseille, France) and cultured in the above growth medium. The purpose of using this medium was to show that extrinsic signals are not required for transdifferentiation when cells are transfected with FL-HNF4α. Similarly, 1 x 10^5 Lin OSMRβ+ cells were initially cultured for 2 days in the above differentiation medium and then transfected with 1 μg of the DN-HNF4α construct using the Magnetofection reagents (OZ Biosciences, Marseille, France). After 24 h of transfection, cells were cultured for another 5 days in the
respective medium containing 15 µg/ml G418. Empty vector (EV)-transfected cells were used as a negative control.

**BrdU incorporation**- Cells were given a pulse of 10 μM bromodeoxyuridine (BrdU) for 2 h prior to termination of the experiments. Cells were fixed with 4% paraformaldehyde in PBS for 30 min and then treated with 2 N HCl at 37°C for 20 min. The acidic pH of the solution was neutralized with borate buffer. Nuclear incorporation of BrdU was determined by immunostaining with an anti-BrdU antibody at 1:200 (eBiosciences). Cells were imaged under an Olympus fluorescence microscope. The percentage of proliferating cells was calculated by counting the BrdU-incorporated nuclei in an average of 10 fields per culture well.

**Immunocytochemistry**- Fixed and permeabilized (Supplementary Methods) cells were stained with goat anti-mouse albumin (Bethyl, Montgomery, TX), anti-CK-18, anti-α-fetoprotein, anti-HNF4α (all from Santa Cruz Biotechnology Inc., Santa Cruz, CA), anti-BrdU (eBiosciences, CA) and anti-flag (Sigma-Aldrich Corporation, St. Louis, MI) antibodies at room temperature for 1-2 h. For surface molecules, cells were labeled with anti-CD45 (BD Pharmingen, San Jose, CA), anti-c-Met and anti-OSMRβ (R&D Systems, Minneapolis, MN) antibodies. AlexaFluor488/594/633-conjugated streptavidin or secondary antibodies (Molecular Probes Inc., OR) were used to identify the specific proteins. As negative controls, isotype antibodies/fluorophore-conjugated secondary antibodies alone were also used. The nuclei were stained with 4′,6-Diamidino-2-phenylindole (DAPI) or propidium iodide (PI). Cells were imaged with an Olympus fluorescence microscope using the LCPlanFl 20x and 40x objectives, an Olympus Fluoview1000 confocal laser scanning microscope using a PlanApomat 60x oil immersion objective and a DP70 digital camera. The Olysia Bioreport and FlowView 5.0 software were used for image acquisition. Images were composed and edited in Image ProPlus and Photoshop 6.0 (Adobe).

**Cytochrome p450 activity**- The functional activity of 32D cell-derived hepatocytes was assessed by *in situ* formation of resorufin from pentoxyresorufin (23).

**RT-PCR**- Details of the total RNA extraction and the analysis of the amplified products are given in the Supplemental Methods (18). The primers and amplification conditions for the PCR reactions are shown in Supplemental Table 1.

**Flow cytometry and western blotting**- Details of the flow cytometry (18) and western blotting (30) are mentioned in the Supplemental Methods.

**Statistical analysis**- Results of multiple experiments are reported as the means ± SEM (Standard Error of the Mean). One-way analysis of variance (ANOVA) was used to calculate the significance between two means.

**RESULTS**

**Kinetics of differentiation of Lin−OSMRβ+ cells into hepatocytes**. Clonal culture of Lin−OSMRβ+ cells was conducted to study the kinetics of differentiation. The cumulative results of two independent experiments are shown in Fig. 1A. The expression of albumin and complete down-regulation of a common leukocyte antigen (CD45) were considered as indicators of hepatic differentiation. Initially, most of the cells expressed CD45 (Supplemental Fig. 1A & 1B); no hepatic differentiation was apparent until day 3 of the culture (Fig. 1A & Supplemental Fig. 1B). Cellular changes were first observed at day 5 of the culture (Supplemental Fig. 1B). The lineage switch was observed on 7 day, which was associated with morphological changes and cellular proliferation (Fig. 1A & Supplemental Fig. 1B). About 70% of the Lin′OSMRβ+ cells differentiated into albumin-expressing hepatic cells after 10 days of culture (Fig. 1A).

Roles of OSMRβ and c-Met have been documented in hepatic differentiation, cellular maturation and proliferation during liver development (31-33). In this study, we intended to examine the expression of these receptors by immunocytochemical analysis. Although we sorted Lin′OSMRβ+ cells, the initial expression of OSMRβ was low (Fig. 1B). In addition, c-Met was not expressed until day 3 of the culture.
(Fig. 1B). OSMRβ expression was enhanced on day 3 and continued until day 5 of the culture. The expression of albumin increased on day 5, when both of the receptors were highly expressed. After 5 days of culture, OSMRβ expression was completely suppressed (Fig. 1B).

HNF4α is activated during culture of cells. We stained the cells with anti-HNF1α and anti-HNF4α antibodies after 7 days of culture. HNF4α was detected in the nuclei of the albumin-expressing cells (Supplemental Fig. 2). Since OSM is one of the components of DLS-albumin-expressing cells (Supplemental Fig. 2), we neutralized it and examined the nuclear localization of HNF4α. OSM neutralization caused declines in both expression and nuclear localization of HNF4α (Fig. 2A). Quantification of HNF4α* nuclei revealed that OSM neutralization abrogated HNF4α activation in 54 ± 9% (n = 3) of cells compared to the control experiment. To determine the effect of OSM neutralization on differentiation, cells were analyzed by flow cytometry for the expression of albumin. In OSM-neutralized cultures, 67% of cells did not express albumin compared to the control (Fig. 2B). In the subsequent experiments, we examined the time course of expression and nuclear localization of HNF4α by western blot analysis. HNF4α was first detected in the cytoplasm on day 5 of the culture, which was 50-fold higher than that present on day 3 (Fig. 2C, Supplemental Table 2). Nuclear localization of HNF4α was detected on day 7 of culture. These results were compared with a immunocytochemical analysis run in parallel experiments. After 3 days of culture, HNF4α was detected in the cytoplasm, but albumin was not expressed by the cells. The synthesis of albumin was associated with higher expression of HNF4α and its nuclear localization at day 5 of the culture (Fig. 2D, middle panel). Further expression of HNF4α and its nuclear translocation augmented albumin synthesis in the later time points (Fig. 2D, lower panel). The differences in the expression pattern of HNF4α in the above two experiments was due to the sensitivity of the assay methods. The expression of HNF4α, its nuclear localization and the synthesis of albumin indicated that these events were linked. These results also suggested that HNF4α induction is one of the downstream events of the differentiation program and that the OSM/OSMRβ signaling pathway may play a role.

Ectopic expression of HNF4α induces transdifferentiation of 32D cells. The results of earlier experiments suggested that HNF4α is involved in the transdifferentiation of Lin’ OSMRβ cells into hepatocytes. Whether HNF4α alone was able to cause these cellular changes was not clear. To answer this question, we transfected 32D cells with the FL-HNF4α construct. As a control, empty vector (EV) was used to transfected the same cells. A distinct morphology resembling epithelial cells was observed in HNF4α-transfected cells compared with the EV-transfected cells (Supplemental Fig. 3A). The expression of CD45 and OSMRβ was suppressed in more than 80% of the transfected cells as compared to the wild type 32D cells (Supplemental Fig. 3B1 & 3B2). The expression of c-Met, a characteristic receptor of hepatocytes, was examined in these transfected cells. c-Met was expressed (Fig. 3A:b) in all of the surviving transfected 32D cells; it was absent prior to the transfection (Supplemental Fig. 3C). The biological function of c-Met was tested by pulsing cells with BrdU in the absence or presence of HGF. About 70% of the cells cultured in the presence of HGF incorporated BrdU into their nuclei, and this number was lower in the absence of HGF (Fig. 3A- h,e). The incorporation of BrdU in nuclei of the HGF-induced culture suggested that the c-Met expressed in 32D cells was functional.

HNF4α expression is essential for liver development; it acts upstream of the transcription factors HNF1α and pregnane-x-receptor (PXR) (34). To examine the regulation of ectopically expressed HNF4α using a few downstream genes, we performed RT-PCR analysis of 32D cells transfected with EV and FL-HNF4α. The HNF1α and albumin genes were transcribed in cells over-expressing HNF4α, indicating that these are downstream target genes (Fig. 3B). HNF3β and c/EBPα were not expressed in the induced hepatocytes, which have been reported to be members of the transcription factors network involved in liver
development. Interestingly the hematopoietic lineage transcription factor GATA-1 was completely suppressed in 32D cells that over-expressed HNF4α.

Next, we examined the loss of myeloid colony-forming activity in these cells. The myeloid colony-forming ability of the HNF4α transfected cells was significantly \((p < 0.005, n = 3)\) lower compared with the EV-transfected cells (Fig. 3C).

Induced hepatocytes were also analyzed for the expression of hepatic markers and functional activity. As expected, EV-transfected cells did not show albumin expression (Supplemental Fig. 4). In the presence of G418, only the FL-HNF4α transfectants survived, and all of their nuclei were HNF4α+ (Fig. 4b,4f,4j). Nevertheless, the transfectants expressed the hepatic markers albumin (Fig. 4c), α-fetoprotein (Fig. 4g) and CK-18 (Fig. 4k). Besides the expression of hepatic markers, the transfected cells were found to be functionally active. The metabolic enzyme p450 was active because these cells metabolized 7-pentoxyresorufin into resorufin (Fig. 4n). The presence of hepatic markers, functional activity and mitogenic response to HGF suggested that ectopic expression of HNF4α caused transdifferentiation of 32D cells into hepatocyte-like cells.

**Ectopic expression of DN-HNF4α inhibits transdifferentiation of LinOSMRβ+ cells.** The experiments above confirmed that HNF4α induces transdifferentiation of hematopoietic cells into hepatocytes, but it was not known whether the transcription factor was indispensable for this process. Therefore the Lin'OSMRβ+ cells were transfected with a DN-HNF4α gene during hepatic differentiation. DN-HNF4α neutralizes the effects of endogenous and ectopically expressed FL-HNF4α (29). Previously we showed that about 80% of Lin'OSMRβ+ cells differentiated into albumin- and CK-18-expressing hepatic cells (23). After 5 days of culture, cells were analyzed for expression of the albumin and tyrosine aminotransferase (TAT) genes by semi-quantitative RT-PCR. The DN-HNF4α transfectants expressed considerably lower amounts of albumin and TAT mRNA, as compared with the EV-transfected cells (Fig. 5). The suppression of hepatic genes in DN-HNF4α-transfected Lin'OSMRβ+ cells confirmed the obligatory role of HNF4α during transdifferentiation.

**DISCUSSION**

There are many reports showing that BM-derived stem cells can differentiate into cells of other germ lines (6,7). It is now known that transdifferentiation may occur in many physiological processes, for example in development and regeneration (35-37). Since transdifferentiation is thought to be one of the mechanisms for tissue regeneration, we sought to determine the molecular mechanism for conversion of BM-derived stem cells into hepatocytes. In this study, we have shown that HNF4α acts as a molecular switch for transdifferentiation of putative competent cells from bone marrow into hepatocytes.

It has been shown previously that both OSMRβ and c-Met are involved in the maturation of hepatocytes during liver development, but their functions at the molecular levels are different (38). The function of OSM starts at an early stage of the fetal liver and continues up to the late stages of liver development. In contrast, HGF is involved in neonatal hepatic maturation after the level of OSM in the fetal liver microenvironment has declined (38). This report indicated that the biological roles of OSM and HGF are distinct. We found that OSMRβ expression was suppressed after 5 days of culture, whereas c-Met expression continued. Again, c-Met was not expressed at the beginning of the culture. Together, these results suggested that these signaling pathways have distinct roles at the initial phase of transdifferentiation. The expression of HNF4α as well as its nuclear localization was partially suppressed due to neutralization of OSM. This was also associated with a decrease in the albumin-expressing cells, suggesting that HNF4α expression is a downstream effect of the OSM/OSMRβ signaling pathway. Previous reports showed that OSM treatment in mouse and human fetal hepatocytes enhances HNF4α
expression (39,40). Thus our results implicated OSM in the differentiation of Lin-OSMRβ+ cells into hepatocytes. However, this study does not exclude the potential role of other growth factors present in the DLS.

Many functional activities of hepatocytes are lost in HNF4α-/- fetal liver cells (38). Furthermore, its conditional inactivation diminishes the expression of many regulatory hepatic transcription factors involved in liver development (37). Incidentally, we observed persistent expression of HNF4α during the culture. The liver-enriched transcription factor C/EBPα is involved in the transition of albumin-negative hepatic stem cells to albumin-positive cells in the presence of HGF (41). Consistent with our previous report (23), here also we could not detect the C/EBPα transcript, confirming that the transcriptional network for transdifferentiation and liver development are not the same. It has been proposed that during liver development individual promoters and enhancers possess binding sites for multiple transcription factors, which may act coordinately to regulate expression of a large array of genes. This suggests that the function of one transcription factor may be compensated by another (35).

Ligand-dependent experiments confirmed that hematopoietic cells can be converted into hepatocytes, provided that the cells are exposed to the transdifferentiating culture environment. The study also showed that HNF4α was clearly expressed in the differentiating cells. However, it was not apparent whether HNF4α expression caused transdifferentiation of the hematopoietic stem cells into hepatocytes. To examine the role of ectopic expression of HNF4α, we adopted a ligand-independent culture process in which 32D cells were transfected with the target gene. With regard to HNF4α, the results of both ligand-dependent and ligand-independent experiments were comparable because the differentiated cells expressed albumin, CK-18 and other hepatic genes. In addition to these hepatic genes, expression of the hematopoietic transcription factor GATA1 was completely abolished, and the cells acquired the functional receptor for HGF (c-Met). Intriguingly, HNF4α transfection induced the expression of one of its downstream transcription factors, HNF1α, which is supposed to be involved in liver development (35). Another important downstream transcription factor, PXR, was regulated by HNF4α. PXR is known to be involved in the expression of CYP genes (40). By indirect observation, we confirmed the transcription of PXR, since induced differentiation resulted in the expression of active cytochrome p450 enzyme in hepatic cells. We also showed that DN-HNF4α suppressed transdifferentiation of Lin-OSMRβ+ cells because expression of both albumin and TAT genes were decreased. The partial effect of the DN plasmid on hepatic gene expression was due to low transfection efficiency in the case of primary cells. It has been shown that an excess (80-fold) of DN-HNF4α plasmid expression is required to abolish the effect of the FL plasmid (29). Overall, the effects of ectopic expression of a single transcription factor, HNF4α, in 32D cells were consistent with its role in transdifferentiation of Lin-OSMRβ+ cells into hepatocytes.

In summary, we propose that transdifferentiation of hematopoietic cells into hepatocytes involves induction and activation of HNF4α. The cellular and molecular changes in hematopoietic cells into hepatocytes were shown by the expression of hepatocyte-specific genes, proteins and functional activity. HNF4α was found to be indispensable for the manifestation hepatic characteristics because transdifferentiation depends on its activation (ligand-dependent or induced expression); conversely, dominant-negative HNF4α inhibited transdifferentiation. In this study, we did not examine cell fusion because no cells other than hematopoietic cells were used.
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**FOOTNOTES**

Authors are indebted to Prof. Connie Eaves, Terry Fox Laboratory, British Columbia Cancer Research Center, Vancouver, Canada for providing the 32Dcl3 cell line and Dr. Todd Leff, Wayne State University School of Medicine, Detroit, MI for providing the full-length and dominant-negative constructs of the HNF4α gene.

**FIGURE LEGENDS**

**Fig. 1.** Kinetics of differentiation of BMCs into hepatic cells. Lin−OSMRβ+ cells (1 cell/well) were cultured in 96-well plates for different times in the presence of transdifferentiation medium (Supplemental Methods). One half of the medium was replaced twice in a week. Twenty-five wells for each time point were examined. A. Quantitative analysis (immunocytochemistry based) of cells expressing CD45, CD45 and albumin or albumin. Red bars: CD45+Alb− cells; Yellow bars: CD45’Alb’ cells; Green bars: CD45’Alb’ cells. Results show that hematopoietic cells were transdifferentiated into albumin-expressing hepatic cells after 7 days of culture. Note that the results of all of the wells were not reported, as a few wells in each time point showed either no cells or no staining with the CD45/Alb antibodies. B. Confocal microscopic analysis of OSMRβ, c-Met and albumin. Representative photomicrographs show that OSMRβ was initially expressed in the cells but not c-Met. After 5 days of culture, cells expressed OSMRβ, c-Met and albumin. After day 5, the expression of OSMRβ was completely down-regulated. The number of experiments (n) was 2.

**Fig. 2.** Time course of expression and nuclear localization of HNF4α. Lin−OSMRβ+ cells were cultured in the presence of transdifferentiation medium. The expression and nuclear localization of HNF4α were analyzed at different times. A. Immunocytochemical analysis of HNF4α in OSM-neutralized culture. Cells were cultured without or with OSM-neutralizing
antibody. Control cells show the expression of HNF4α in the majority of the cells (nuclei stained with DAPI are pseudocolored in red) (left panel). HNF4α expression and nuclear localization were reduced in the presence of the OSM-neutralizing antibody (right panel) (n = 3). B. Flow cytometric analysis of albumin expression. The bar diagram shows amount of albumin-expressing cells in the respective cultures. The cellular differentiation process was suppressed (green bar) in OSM-neutralized medium compared with the DLS control culture (black bar). The red bar (background reading) indicates cells that were cultured in the normal serum (NS) supplemented medium (n = 3). C. Western blot analysis of HNF4α expression. Cytoplasmic extract (CE) and nuclear extract (NE) of cells at different time intervals were probed with anti-HNF4α, anti-actin and anti-Histone H3 antibodies. A strong signal for nuclear localization of HNF4α was observed after 7 days of culture. Fold expression/activation of HNF4α are shown as normalized values with respect to cytoplasmic and nuclear proteins (Supplemental Table 2). HNF4α, actin and histone H3 migrate as 55, 43 and 17 kDa proteins, respectively. D. Immunocytochemical analysis of nuclear localization of HNF4α. Representative confocal photomicrographs show that HNF4α was expressed from day 3 and increased on day 5 of culture. High expression of albumin was observed on day 7 of culture. Arrows indicate nuclear localization of HNF4α.

Fig. 3. Ectopic expression of HNF4α induces differentiation of 32D cells. The transfected cells were analyzed for the expression of cell surface receptors, colony-forming ability and gene expression. A. The functional activation of c-Met in FL-HNF4α-transfected cells. In the transfected cells that expressed c-Met (b), the number of BrdU-incorporated cells was lower in the absence of HGF (e) than in its presence (h) (n = 3). B. RT-PCR analysis of gene expression in FL-HNF4α-transfected cells. The HNF1α, HNF4α and albumin genes were expressed in the transfected cells, whereas GATA-1 was completely down-regulated. AML-12 (mouse primary hepatocytes) was used as a positive control for hepatic genes (n = 2). HNF4α, HNF1α, HNF3β, c/EBPα, GATA1, albumin and GAPDH migrate as 180, 187, 216, 314, 187, 465 and 209 bp DNA fragments, respectively. C. Myeloid colony assay (42). HNF4α transfection caused a significant drop in colony-forming potential of the cells (n = 3).

Fig. 4. HNF4α–transfected 32D cells synthesize hepatocyte-specific proteins. HNF4α and hepatic proteins were analyzed by immunocytochemistry using specific antibodies. Representative micrographs show the presence of HNF4α in the nuclei of cells (b,f,j) that express albumin (c), α-fetoprotein (g) and CK-18 (k). The induced, differentiated cells expressed the p450 enzyme because the fluorescent compound resorufin (n) was produced from 7-pentoxyresorufin (n = 3).

Fig. 5. Ectopic expression of DN-HNF4α in Lin-OSMRβ cells inhibits transdifferentiation. Lin-OSMRβ cells were cultured and transfected with the EV or DN-HNF4α plasmids for 7 days. Comparative mRNA expression of albumin (left) and TAT (right) are shown in representative agarose gel pictures. Lane 1: Purified Lin-OSMRβ cells (negative control); Lane 2: Lin-OSMRβ cells transfected with EV (vector control); Lane 3: Lin-OSMRβ cells transfected with the DN-HNF4α plasmid (test sample); Lane 4: AML-12 cells (positive control). Albumin, TAT and GAPDH migrate as 465, 422 and 209 bp DNA fragments, respectively. Intensities of respective bands were quantified with the Image J software. The background intensity in the first lane was subtracted from that of the other three lanes. The absolute readings were normalized with respect to the GAPDH band. Bar diagrams show the relative transcription levels of the albumin and TAT genes (n = 2).
**Figure 1**

**A**

![Bar chart showing the number of wells with different time points and cell markers.]

**B**

![Images showing immunofluorescence staining for different time points and cell markers.]

- **Day 1:** OSMRβ, c-Met, Albumin, Merge
- **Day 3:** OSMRβ, c-Met, Albumin, Merge
- **Day 5:** OSMRβ, c-Met, Albumin, Merge
- **Day 7:** OSMRβ, c-Met, Albumin, Merge

**Legend:**
- CD45 Alb⁺
- CD45 Alb⁻
Figure 2

A. Control vs. OSM-neutralized Albumin expressing cells (x10^4)

B. Bar graph showing the number of Albumin expressing cells (x10^4)

C. Table showing the expression levels of HNF-4α, Actin, and Histone over 3, 5, and 7 days with NE and CE conditions.

D. Immunofluorescence images of PI, HNF4α, Albumin, and Merge for 3, 5, and 7 days.
Figure 3

A

- HGF
- FLAG
- BrdU
- Merge

+ HGF
- FLAG
- BrdU
- Merge

100 μ

B

EV
FL
AML-12

HNF4α
HNF1α
HNF3β
C/EBPα
GATA1
Albumin
GAPDH

C

Colony/10,000 cells

EV
FL

p < 0.005
Figure 4
Figure 5
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J. Biol. Chem. published online December 16, 2009

Access the most updated version of this article at doi: 10.1074/jbc.M109.058198

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