HES-1 Is Involved in Adaptation of Adult Human β-Cells to Proliferation In Vitro

Yael Bar, Holger A. Russ, Sarah Knoller, Limor Ouziel-Yahalom, and Shimon Efrat

OBJECTIVE—In vitro expansion of β-cells from adult human islets could solve the tissue shortage for cell replacement therapy of diabetes. Culture of human islet cells typically results in <16 cell doublings and loss of insulin expression. Using cell lineage tracing, we demonstrated that the expanded cell population included cells derived from β-cells. Understanding the molecular mechanisms involved in β-cell fate in vitro is crucial for optimizing expansion and redifferentiation of these cells. In the developing pancreas, important cell-fate decisions are regulated by NOTCH receptors, which signal through the hairy and enhancer of split (HES)-1 transcriptional regulator. Here, we investigated the role of the NOTCH signaling pathway in β-cell dedifferentiation and proliferation in vitro.

RESEARCH DESIGN AND METHODS—Isolated human islets were dissociated into single cells. β-Cells were genetically labeled using a Cre-lox system delivered by lentviruses. Cells were analyzed for changes in expression of components of the NOTCH pathway during the initial weeks in culture. HES-1 expression was inhibited by a small hairpin RNA (shRNA), and the effects on β-cell phenotype were analyzed.

RESULTS—Human β-cell dedifferentiation and entrance into the cell cycle in vitro correlated with activation of the NOTCH pathway and downregulation of the cell cycle inhibitor p57. Inhibition of HES-1 expression using shRNA resulted in significantly reduced β-cell replication and dedifferentiation.

CONCLUSIONS—These findings demonstrate that the NOTCH pathway is involved in determining β-cell fate in vitro and suggest possible molecular targets for induction of β-cell redifferentiation following in vitro expansion. Diabetes 57:2413–2420, 2008

Replacement of β-cells by transplantation is a promising approach for treatment of type 1 diabetes; however, its application on a large scale is limited by the availability of pancreas donors. In a normal adult pancreas, a slow rate of β-cell renewal is responsible for maintenance of an adequate functional β-cell mass. This rate is accelerated in conditions of increased demands for insulin, such as pregnancy (1) and obesity (2). Work in an animal model demonstrated that new β-cells are generated in adult mice predominantly by replication of pre-existing β-cells rather than by neogenesis from insulin-negative stem/progenitor cells (3). This finding has raised hopes for recapitulation of β-cell expansion in cultures of adult human islets. However, previous attempts at in vitro expansion of adult human β-cells resulted in a limited number of cell population doublings and loss of insulin expression (4–7). Insulin-negative cells with a considerable proliferative capacity were derived from cultured human islets (8–10). Insulin expression in these cells could be induced by changing the culture conditions; however, its levels were low and varied among donors (8–10). One possible interpretation of these results is that β-cells survive, dedifferentiate, and divide in culture. Recently, we applied a genetic cell lineage tracing approach for labeling cultured adult human islets and demonstrated that, in contrast to mouse β-cells (11–14), dedifferentiated, label-positive cells derived from human β-cells could be induced to significantly proliferate in vitro (15). These cells may be of value for development of cell therapy for type 1 diabetes, since they may retain some β-cell-specific chromatin structure to allow their redifferentiation. Understanding the molecular mechanisms involved in β-cell dedifferentiation and replication in vitro may facilitate the expansion and redifferentiation of these cells.

In the developing pancreas, important cell-fate decisions, including the switch from proliferation to differentiation, and the choice between exocrine and endocrine fates (16), as well as among different endocrine fates (17–19), are regulated by the NOTCH signaling pathway. Expression of NOTCH ligands on a differentiating cell inhibits development of the same phenotype in neighboring cells, in a mechanism termed lateral inhibition (16). Ligand binding to NOTCH receptors on a neighboring cell results in cleavage of the NOTCH intracellular domain (NICD), which enters the nucleus and forms a complex that modulates gene expression (20). The hairy and enhancer of split (HES) family of transcriptional regulators is a major target of the NICD complex. In fetal pancreata, HES-1 acts as an inhibitor of neurogenin 3 (NGN3) gene expression, which is required for islet development (21). In addition, HES-1 regulates the cell cycle by inhibiting expression of genes encoding the cyclin kinase inhibitors p27 and p57 (22,23). Some evidence suggests that it may also inhibit insulin gene expression (17). Overall, HES-1 is associated with promoting cell replication and preventing cell differentiation. Forced expression of NOTCH inhibits pancreas cell differentiation (17,18), while mice with null mutations in genes encoding NOTCH pathway components exhibit accelerated differentiation of endocrine pancreas (16,21). The NOTCH pathway is not normally expressed in the adult pancreas; however, it is activated in conditions associated with cell dedifferentiation and replication, such as regeneration following experimental pancreaticitis (24), pancreatic neoplasia (25), metaplasia of cultured pancreatic exocrine cells (26), and in rat β-cells exposed to cytokines (27).
We hypothesized that β-cell dedifferentiation and entrance into the cell cycle in vitro involve induction of the NOTCH pathway. Our findings demonstrate a considerable activation of the NOTCH pathway in these cells, which correlates with downregulation of the cell cycle inhibitor p57 and loss of insulin expression. Inhibition of HES-1 expression using small hairpin RNA (shRNA) results in reduced replication of cultured β-cells and a decrease in cell dedifferentiation. These findings suggest possible molecular targets for prevention of β-cell dedifferentiation in culture or induction of cell redifferentiation following in vitro expansion.

RESEARCH DESIGN AND METHODS

Islet cell culture. Islets were received 2–3 days following isolation. Islets from individual donors were dissociated into single cells and cultured in CMRL-1066 medium containing 5.6 mmol/L glucose and supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, 100 μg/ml streptomycin, 100 μg/ml gentamicin, and 5 μg/ml amphotericin B as described (8). The cultures were refed twice a week and split 1:2 once a week.

HES-1 inhibition and lineage tracing. HES1 shRNAs (accession nos. TRCN18898, 18899, 18900, 18901, and 18993) and a non-target shRNA cloned in pLKO.1 lentiviral vector, were obtained from the RNAi Consortium (Sigma-Aldrich). Virus was produced in 293T cells following cotransfection with the pCMV-DdR8.91 and pM2D2.G packaging plasmids. The culture medium was harvested 48 h later. Islet cells cultured for 1–2 days were washed with PBS and infected at multiplicity of infection 2:5:1 in CMRL-1066 medium containing 8 μg/ml polybrene overnight. The medium was then replaced with regular culture medium. Four days after infection, the cells were selected for puromycin resistance (1 μg/ml) for 3 days. Two weeks after infection, the cells were harvested for further analysis. For γ-secretase inhibition, 5 μmol/L of L-685,458 (Calbiochem) were added to cells at passage 2 (P2) for 17 h. Lineage tracing was performed using the RIP-Cre and pTrip cytomegalovirus (CMV)-loxp-P-DsRed2-loxP-eGFP at a total multiplicity of infection 4:1. The cDNA levels were normalized to human ribosomal protein P0 cDNA.

Apoptosis assay. Terminal uridine deoxynucleotidyl transferase dUTP nick-end labeling assay was performed using a Chemicon ApopTag Fluorescein In Situ Apoptosis Detection Kit according to the instructions of the manufacturer. Cells at P0 were stained 24 h following culture initiation. Cells were stained for insulin as described above.

Statistical analysis. Significance was determined using Student’s t test. To approach a normal distribution of the qPCR data, a logarithmic transformation was performed. To account for multiple testing, the Bonferroni correction was applied.

RESULTS

Upregulation of HES-1 in cultured human β-cells. Human islets were isolated from nine donors, six males and three females aged 38–60 years (mean age 50 ± 8), with a purity ranging between 65 and 85% (mean 78 ± 6%). Islets from each donor were dissociated and expanded in culture as described (8). Quantitative RT-PCR (qPCR) analyses of RNA extracted from these cells during the first 2 weeks of culture revealed a rapid dedifferentiation, as previously reported (8), which was manifested in a drastic decrease in insulin mRNA levels, averaging 166-fold (P = 8.7 × 10−7 at P2) (Fig. 1A) (see data on cells from individual donors in online appendix Fig. 1 [available at http://dx.doi.org/10.2337/db07-1323]). Concomitant with this decrease, an increase in HES1 mRNA was observed in cells from all donors, averaging 4.6-fold (P = 0.006 at P2) within the first 2 weeks of culture (Fig. 1A). A similar increase was noted in HES1-protein levels (Fig. 1B). At both RNA and protein levels, the wave of HES-1 upregulation peaked within the first 2 weeks of culture and was downregulated thereafter. To demonstrate that HES-1 upregulation in this system was dependent on NOTCH activation, we used an inhibitor of γ-secretase, the protease complex required for generation of NICD (30). As seen in Fig. 1C, cell incubation with the inhibitor resulted in 10-fold lower HES-1 levels, supporting a NOTCH-dependent mechanism. Immunostaining could not detect significant HES-1 expression in nuclei of cells with intense

### Table 1

| Gene   | Probe         |
|--------|---------------|
| DLL1   | Hs00194509_m1 |
| HES1   | Hs00172878_m1 |
| Insulin| Hs00355773_m1 |
| JAG2   | Hs00171432_m1 |
| NOTCH1 | Hs00413187_m1 |
| NOTCH2 | Hs01050706_m1 |
| NOTCH3 | Hs01128547_m1 |
| NOTCH4 | Hs00965897_m1 |
| p57    | Hs00197583_m1 |
| PDX1   | Hs00436216_m1 |
| RPLP0  | Hs00990902_m1 |
insulin staining (Fig. 1D). In contrast, HES-1 was clearly detected in insulin-negative cells. To monitor HES-1 expression in dedifferentiated cells derived from β-cells, β-cells in freshly isolated islets were heritably labeled using a cell lineage tracing approach recently developed in our laboratory (15). The labeling approach is based on cell infection with a mixture of two lentivirus vectors: one expressing Cre recombinase under the insulin promoter (RIP-Cre) and the other a reporter cassette in which the CMV promoter is separated from an eGFP gene by a loxP-flanked stop region. Removal of the stop region in β-cells infected by both viruses activates eGFP expression specifically in these cells, thereby allowing continuous tracking of β-cell fate after insulin expression is lost. Residual insulin expression in β-cells during the initial days in culture provides a sufficient window of time for RIP-Cre expression and eGFP activation. Using this method, 57.5 ± 8.9% of insulin-positive cells were labeled with eGFP (15). Analysis of the cells expanded in culture following labeling revealed HES-1 staining in cells that lost insulin expression but maintained eGFP expression, demonstrating that they were derived from β-cells (Fig. 1D). A total of 89.3 ± 0.1% of eGFP+/insulin-negative cells were HES-1+ (based on counting >200 cells in cultures from each of three donors).

**Changes in expression of components of the NOTCH pathway in cultured human β-cells.** qPCR analyses revealed changes in levels of transcripts encoding the four members of the NOTCH family. NOTCH1 transcripts were upregulated on average by 3.6-fold within the first 2 weeks of culture (P = 0.02 at P2) (Fig. 2A). A similar increase was detected in the NOTCH1 120-kd transmembrane fragment (Fig. 2B), paralleling the changes in HES-1 levels (Fig. 1B). NOTCH2 and NOTCH3 were significantly upregulated on average by 9.7-fold (P = 8.8 × 10−5 at P2) and 10.1-fold (P = 1.0 × 10−4 at P2), respectively, within the first 2 weeks of culture. Overall, the activation of NOTCH1–3 paralleled that of HES-1. In contrast, NOTCH4 was drastically downregulated, on average 50-fold (P = 3.0 × 10−5 at P2) from its level in primary islets. As with HES1 upregulation, NOTCH1–3 upregulation peaked within the first 2 weeks of culture and was downregulated thereafter. Transcripts encoding presenilin-1, a component of the γ-secretase complex, and recombination signal-binding protein 1 for Jκ (RBPJK), a protein that participates in the NICD nuclear complex, were not significantly changed (data not shown). In contrast, transcripts for NOTCH ligands were downregulated during the initial weeks of culture (Fig. 2A). DELTA1 transcripts were downregulated on average 3.3-fold (P = 1.4 × 10−4 at P2) within the first 2 weeks of culture. JAG1 transcripts were not significantly changed (data not shown). JAG2 transcripts were downregulated on average 5.5-fold (P = 1.9 × 10−5 at P2) within the first 2 weeks of culture. The increased activity of the NOTCH pathway was manifested by appearance of NICD in cell nuclei, as revealed by immunostaining (Fig. 2C). Similar to the pattern of HES1 immunostaining, staining for NICD could not be detected in cells intensely stained for insulin. NICD staining was detected in all (100%) lineage-labeled, insulin-negative cells identified as originating from β-cells by eGFP expression (Fig. 2C) (based on counting >200 cells in cultures from each of three donors).

**Changes in expression of cell cycle inhibitors.** To evaluate the consequences of increased HES-1 expression in the cultured islet cells, we analyzed changes in transcripts of genes encoding cyclin kinase inhibitors, which are among the main targets of repression by HES-1 (22,23). Transcripts encoding p57, which is thought to be the main cell cycle inhibitor in human β-cells (22), were downregulated, on average, 2.9-fold (P = 0.004 at P2) within the first 2 weeks of culture (Fig. 3A). A further reduction was observed at P3, averaging 7.1-fold, compared with P0 (P = 0.002). This finding was supported by immunostaining for p57, which showed its presence in all (100%) lineage-
labeled insulin-positive eGFP+ cells and its absence in all (100%) insulin-negative eGFP+ cells (based on counting >200 cells in cultures from each of three donors) (Fig. 3B). In contrast to p57, transcripts for p21 were upregulated in cells from all donors, and those for p27 varied considerably among donors (data not shown). The down-regulation of p57 transcripts and protein correlated with cell entrance into the cell cycle, as manifested by Ki67 staining in p57-negative eGFP+ cells (Fig. 3C). The nuclear area of replicating cells was 2.5- to 4-fold larger than that of insulin-positive cells, as previously reported (8). The increase in cell size is likely associated with recruitment of quiescent β-cells into the cell cycle (31).

**Inhibition of HES-1 expression prevents induction of β-cell replication.** To further correlate the induction of β-cell replication with HES-1 upregulation, HES-1 induction during the initial weeks of culture was inhibited using shRNA. Following screening of four HES1 shRNA sequences for activity in 293T cells, one of four (TRCN18993) was selected as most efficient, based on reduction in HES-1 protein levels, as analyzed by immunoblotting (data not shown). Isolated human islets were dissociated, and the cells were infected with a lentivirus encoding HES1 shRNA before culture under standard conditions. Selection for drug resistance allowed elimination of uninfected cells. Cells infected with a nontarget shRNA lentivirus and selected under similar conditions served as control. As seen in Fig. 4A, cell infection with the HES1 shRNA virus resulted in up to six-times-lower HES1 protein levels, compared with cells infected with the control virus. The lower HES-1 levels were associated with a diminished cell proliferation compared with cells infected with the control vector, as judged by staining for BrdU incorporation (Fig. 4B). In addition, staining for Ki67 in eGFP+ cells demonstrated a lower replication rate among cells derived from β-cells (Fig. 4B). The reduced replication in cells infected with the HES1 shRNA virus did not correlate with an
increase in cell apoptosis, as judged by immunoblotting analysis for cleaved PARP (Fig. 4C). In addition, terminal uridine deoxyribonucleotidyl transferase dUTP nick-end labeling assay did not detect significant changes between cells at P2 treated with either virus and cells at P0 among insulin-expressing cells or the total cell population (averaging 1.41, 2.19, and 1.64% apoptotic cells among insulin-expressing cells or the total cell population (averaging assay did not detect significant changes between cells following infection with HES1 shRNA TRCN18993 or nontarget virus. β-Actin served as a loading control. B: Top panel: Incidence of BrdU+ cells among cultured islet cells following infection with HES1 shRNA TRCN18993 or nontarget virus. Data are means ± SD (n = 3 donors; >1,000 cells counted in culture from each donor; P = 0.02). Bottom panel: Incidence of Ki67+ cells among eGFP+ cells from two representative donors following infection with HES1 shRNA or nontarget virus. Data are based on >1,000 cells counted in culture from each donor. □, G13; ■, I10. C: Immunoblotting for PARP in protein extracted from islet cells following infection with nontarget (lane 3) or HES1 shRNA TRCN18993 virus (lane 4). Uninfected cells incubated with (lane 1) or without (lane 2) the apoptotic agent staurosporin served as controls. The lower band in lane 1 represents cleaved PARP. β-Actin served as a loading control. D: qPCR analysis of RNA extracted from islet cells following infection with HES1 shRNA TRCN18993 or nontarget virus. RQ indicates relative quantification compared with P0. Data are means ± SD (n = 3 donors). Only the change in p57 is significant (P = 0.001 vs. cells infected with nontarget virus, indicated by star). ■, P0; □, nontarget; ■ HES1 shRNA. All the analyses were done 14 days following viral infection.

To verify that these results were due to specific inhibition of HES1 expression, the studies were reproduced with two additional HES1 shRNAs, TRCN18990 and TRCN18991. As seen in Fig. 7A, cell infection with viruses encoding these shRNAs resulted in reduction in cellular HES1 protein levels. The reduction in HES1 correlated with 5.3-fold (P = 3.3 × 10−5) and 2.2-fold (P = 6.6 × 10−4) higher levels of p57 transcripts in cells expressing the two HES1 shRNAs, respectively, compared with the levels in cells infected with the nontarget virus. The levels of insulin transcripts were 3.4-fold (P = 1.3 × 10−5) and 1.9-fold (P = 2.8 × 10−4) higher, respectively. The levels of PD1 transcripts were 4.0-fold (P = 9.2 × 10−5) and 2.5-fold (P = 1.9 × 10−4) higher, respectively, and the levels of NEUROD1 transcripts were 7.7-fold (P = 3.5 × 10−5) and 3.1-fold (P = 8 × 10−6) higher, respectively. These results are comparable with those obtained using HES1 shRNA TRCN18993, indicating that the effects on cell proliferation and differentiation were caused by specific inhibition of HES1 expression.
Our findings demonstrate that culture of dissociated adult human islet cells in serum-containing medium, which induces β-cell dedifferentiation and replication, involves activation of elements of the NOTCH pathway. Transcript levels for NOTCH1–3 and HES1 are upregulated. In contrast, transcripts for NOTCH4, and the NOTCH ligands DELTA1 and JAG2, are downregulated. These changes were initially observed in a mixed population of islet cells, which likely included contaminating duct and exocrine cells. Using virus-mediated cell lineage tracing, we then determined that these changes occurred in β-cells. The upregulation of the NOTCH pathway correlated with cell dedifferentiation, as manifested by a dramatic decrease in insulin transcripts, and by cell entrance into the cell cycle, as manifested by downregulation of p57 transcripts and an increase in Ki67 staining. The findings at the RNA level

**FIG. 5.** Prevention of HES-1 upregulation by shRNA reduces β-cell dedifferentiation. *A:* qPCR analysis of RNA extracted from islet cells following infection with HES1 shRNA TRCN18993 or nontarget virus. RQ indicates relative quantification compared with P0. Data are means ± SD (n = 3 donors). The changes in all three genes in cells infected with HES1 shRNA, compared with cells infected with nontarget virus, were significant (P < 0.004). ■, P0; □, nontarget; *, HES1 shRNA. *B:* Left: Incidence of insulin-positive cells among cultured islet cells following infection with HES1 shRNA TRCN18993 or nontarget virus. Data are means ± SD (n = 3 donors; >1,000 cells counted in culture from each donor; P = 0.016). Right: Incidence of insulin-positive cells among eGFPβ-cells from two representative donors following infection with HES1 shRNA or nontarget virus. Data are based on >1,000 cells counted in culture from each donor. Significant differences compared with nontarget are indicated by stars. □, G13; ■, H10. *C:* Immunofluorescence analysis of insulin in eGFPβ-cells following infection with HES1 shRNA TRCN18993 or nontarget virus. Bar = 100 µm. All the analyses were done 14 days following viral infection. (Please see http://dx.doi.org/10.2337/db07-1323 for a high-quality digital representation of this figure.)

**DISCUSSION**

Our findings demonstrate that culture of dissociated adult human islet cells in serum-containing medium, which induces β-cell dedifferentiation and replication, involves activation of elements of the NOTCH pathway. Transcript

**FIG. 6.** HES1 shRNA reduces the decrease in insulin content and glucose-stimulated insulin secretion observed during dedifferentiation of cultured islet cells. *A:* Insulin content in cells infected with HES1 shRNA TRCN18993 or nontarget virus compared with primary islets. Data are means ± SD (n = 3 donors). Asterisks indicate statistically significant differences compared with nontarget. *Non-target shRNA.* B: Insulin secretion in response to glucose during a 30-min assay. Data are means ± SD (n = 3 individual experiments from a representative donor). Asterisk indicates statistical significance of the difference between 0 and 16 mmol/l glucose in each cell type (P < 0.04). ■, 0 mmol/l; □, 16 mmol/l.

**FIG. 7.** Effects of HES1 shRNAs TRCN18990 and TRCN18991 on gene expression in cultured islet cells. *A:* Immunoblotting for HES-1 in protein extracted from islet cells 14 days following infection with HES1 shRNA TRCN18990, TRCN18991, or nontarget virus. HSC70 served as a loading control. *B:* qPCR analysis of RNA extracted from islet cells 14 days following infection with HES1 shRNAs or nontarget virus. RQ indicates relative quantification compared with cells infected with nontarget virus. Data are means ± SD (n = 3). Significant differences compared with nontarget are indicated by asterisks. ■, nontarget; □, TRCN18990; *, TRCN18991.
were supported by immunostaining, which demonstrated a negative correlation between the presence of HES-1 or NICD in the nucleus and insulin expression in eGFP cells, which marked their origin from β-cells. These in situ analyses also detected a positive correlation between p57 and insulin expression, confirming the view that β-cell replication involves dedifferentiation.

The key role of HES-1 in these events was revealed by inhibiting its upregulation with shRNA. In these cells, the decrease in p57 was prevented and cell proliferation was greatly reduced. While cell dedifferentiation was not completely prevented, it was significantly inhibited compared with cells in which HES-1 upregulation was not repressed. This was manifested by higher levels of insulin transcripts and fraction of cells immunostaining for insulin, as well as transcripts encoding β-cell transcription factors. In addition, loss of insulin content and secretion was less pronounced. These findings suggest that a partial cell dedifferentiation is independent of HES-1 activity and cell replication; however, induction of advanced dedifferentiation and cell replication requires HES-1 upregulation. This interpretation is supported by the finding that the bulk of decrease in insulin mRNA occurs during the first week, thus preceding the peak in HES-1 mRNA levels. It is therefore possible that loss of most of the insulin content is a precondition for β-cell entrance into cell cycle in vitro.

Given the fact that upregulation of the NOTCH pathway in islet cell cultures followed cell dissociation into single cells, it is unlikely that it was triggered by a cell-associated ligand, as in the lateral inhibition model (16). Rather, it is possible that this pathway is activated in response to soluble serum components, as was demonstrated in a number of cultured cell types (32). This possibility is supported by our findings of decreased expression of NOTCH ligands in islet cell cultures concomitant with HES-1 upregulation. This is reminiscent of the low levels of NOTCH ligands in the embryonic pancreas cells expressing HES-1, which are directed for further proliferation rather than differentiation (16).

Among the four members of the NOTCH family that were analyzed, NOTCH1, NOTCH2, and NOTCH3 transcripts were upregulated, while NOTCH4 transcripts were greatly downregulated. While expression of NOTCH1 and NOTCH2 was implicated in islet development, NOTCH3 and NOTCH4 expression was documented in mesenchymal and endothelial cells (21). Downregulation of NOTCH4 may reflect the elimination of a NOTCH4+ subpopulation, which is present in the original islet cell suspension but for some reason is not maintained in continuous culture.

The wave of HES-1 upregulation peaked within the first 2 weeks of culture and was downregulated thereafter. Nevertheless, the effects of HES-1 were not reversed, as manifested by continuous replication of cells derived from dedifferentiated β-cells for up to 16 population doublings (8,15). The levels of p57 and insulin transcripts did not rebound thereafter, suggesting that their reinduction requires other signals in addition to the decrease in the inhibitory effect of HES-1. This finding suggests a transient role of HES-1 upregulation that is limited to the initial adaptation of islet cells to culture, after which cell replication may continue in the presence of the lower HES-1 levels found in nonreplicating cells.

In summary, our findings provide evidence for activation of the NOTCH pathway in adult cells and offer an in vitro model system for studying interactions within this pathway. In addition, the findings emphasize the role of components of the NOTCH pathway in the transition of quiescent β-cells into a dedifferentiated, proliferative state in vitro. Our findings demonstrate a negative correlation between replication and maintenance of differentiated function in cultured β-cells, suggesting that significant cell expansion inevitably involves dedifferentiation and will require the development of methods for cell redifferentiation following expansion. Components of the NOTCH pathway may represent molecular targets for induction of redifferentiation of the expanded cells.

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