

Regulated and Reversible Induction of Adult Human β -Cell Replication

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Induction of proliferation in adult human β -cells is challenging. It can be accomplished by introduction of cell cycle molecules such as cyclin-dependent kinase 6 (cdk6) and cyclin D₁, but their continuous overexpression raises oncogenic concerns. We attempted to mimic normal, transient, perinatal human β -cell proliferation by delivering these molecules in a regulated and reversible manner. Adult cadaveric islets were transduced with doxycycline (Dox)-inducible adenoviruses expressing cdk6 or cyclin D₁. End points were cdk6/cyclin D₁ expression and human β -cell proliferation, survival, and function. Increasing doses of Dox led to marked dose- and time-related increases in cdk6 and cyclin D₁, accompanied by a 20-fold increase in β -cell proliferation. Notably, Dox withdrawal resulted in a reversal of both cdk6 and cyclin D₁ expression as well as β -cell proliferation. Re-exposure to Dox reinduced both cdk/cyclin expression and proliferation. β -Cell function and survival were not adversely affected. The adenoviral tetracycline (tet)-on system has not been used previously to drive human β -cell proliferation. Human β -cells can be induced to proliferate or arrest in a regulated, reversible manner, temporally and quantitatively mimicking the transient perinatal physiological proliferation that occurs in human β -cells. *Diabetes* 61:418–424, 2012

Human β -cell expansion, in vivo or ex vivo, is an important, but unachieved, goal of diabetes research. This concept drives active research programs in hES and iPS cell differentiation, xenograft sources of islets, expansion and survival of cadaveric human β -cells, and high-throughput small molecule screens, hoping to find approaches that could allow β -cell replacement therapy. Unfortunately, adult human β -cells have proven recalcitrant to induction of proliferation by growth factors, nutrient signaling pathways, and maneuvers such as partial pancreatectomy, induction of insulin resistance, pregnancy, and high-fat feeding (1–7), all of which induce remarkable rodent β -cell proliferation. Thus, investigators are left with replication-recalcitrant adult cadaveric islets as the major starting material for research in β -cell replacement.

We have shown that it is possible to drive adult human β -cells to replicate robustly, using in vitro and in vivo models, by delivery of cell cycle molecules such as cyclin-dependent kinase 6 (cdk6) and cyclin D₁ (8–11). This allows retention of differentiated functions, such as glucose-stimulated insulin secretion, and has no apparent adverse

effects on survival (8–11). A drawback of these studies is that they were performed using continuous overexpression of cdk6 and cyclin D₁ driven by the constitutive cytomegalovirus (CMV) promoter in adenoviral vectors, raising concerns of oncogenic transformation over the long term.

Interestingly, several groups have shown that replication occurs perinatally in the embryonic and neonatal human pancreas for several months, albeit at relatively slow rates (12–14). These observations suggest that strategies that attempt to mimic normal transient perinatal human β -cell proliferation and expansion may have therapeutic application. Thus, in the current study, we asked if adult human β -cell proliferation could be activated in an inducible manner that might also allow restoration of cell cycle arrest (i.e., minimizing oncogenic risk) once a desired β -cell mass had been achieved, all using a temporal profile that resembles events in human pancreas development. Although both cdk6 and cyclin D₁ are individually able to drive human β -cell replication in vitro (9,10), we selected the cdk6 and cyclin D₁ combination for this study, among several cdk-cyclin pair options, because the combination produces greater proliferation than either alone, and because we had tested this combination in vivo in the streptozocin-diabetic NOD-severe combined immunodeficiency model (9,10).

RESEARCH DESIGN AND METHODS

Human islets were obtained from the National Institutes of Health (NIH)- and Juvenile Diabetes Research Foundation (JDRF)-supported Integrated Islet Distribution Program (IIDP) and Dr. Tatsuya Kin (Clinical Islet Laboratory, University of Alberta, Edmonton, Alberta, Canada). Adenovirus preparation, immunoblots, glucose-stimulated insulin secretion, cdk and cyclin expression studies, and proliferation and survival studies were performed as described in detail previously (8–11,15,16) and in the figure legends. Results are expressed as averages \pm SEM. Statistical differences were determined by two-tailed, unpaired Student *t* test or by ANOVA for repeated measures with post hoc analysis, as indicated in the figure legends.

RESULTS

Cdk6 and cyclin D₁ can be overexpressed in a dose-dependent manner in human β -cells transduced with tet-inducible adenoviruses. Human islets were transduced with an adenovirus delivering the tet transactivator (Ad.TTA) and either a control adenovirus expressing green fluorescent protein (Ad.GFP) or adenoviruses expressing cdk6 or cyclin D₁ under the control of the tet response element (Ad.TRE-cdk6 or Ad.TRE-cyclin D₁). Increasing amounts of doxycycline (Dox) (0–1 μ g/mL) were added to the medium to define the dose responsiveness of the cdk6 and cyclin D₁ expression. Figure 1 shows that cyclin D₁ or cdk6, or both, markedly increased with rising concentrations of Dox, plateauing at 0.1 μ g/mL Dox. Close examination indicates that both cdk6 and cyclin D₁ were expressed at low levels in the absence of Dox. For example, control (Ad.GFP-transduced) islets expressed 12.2% of the

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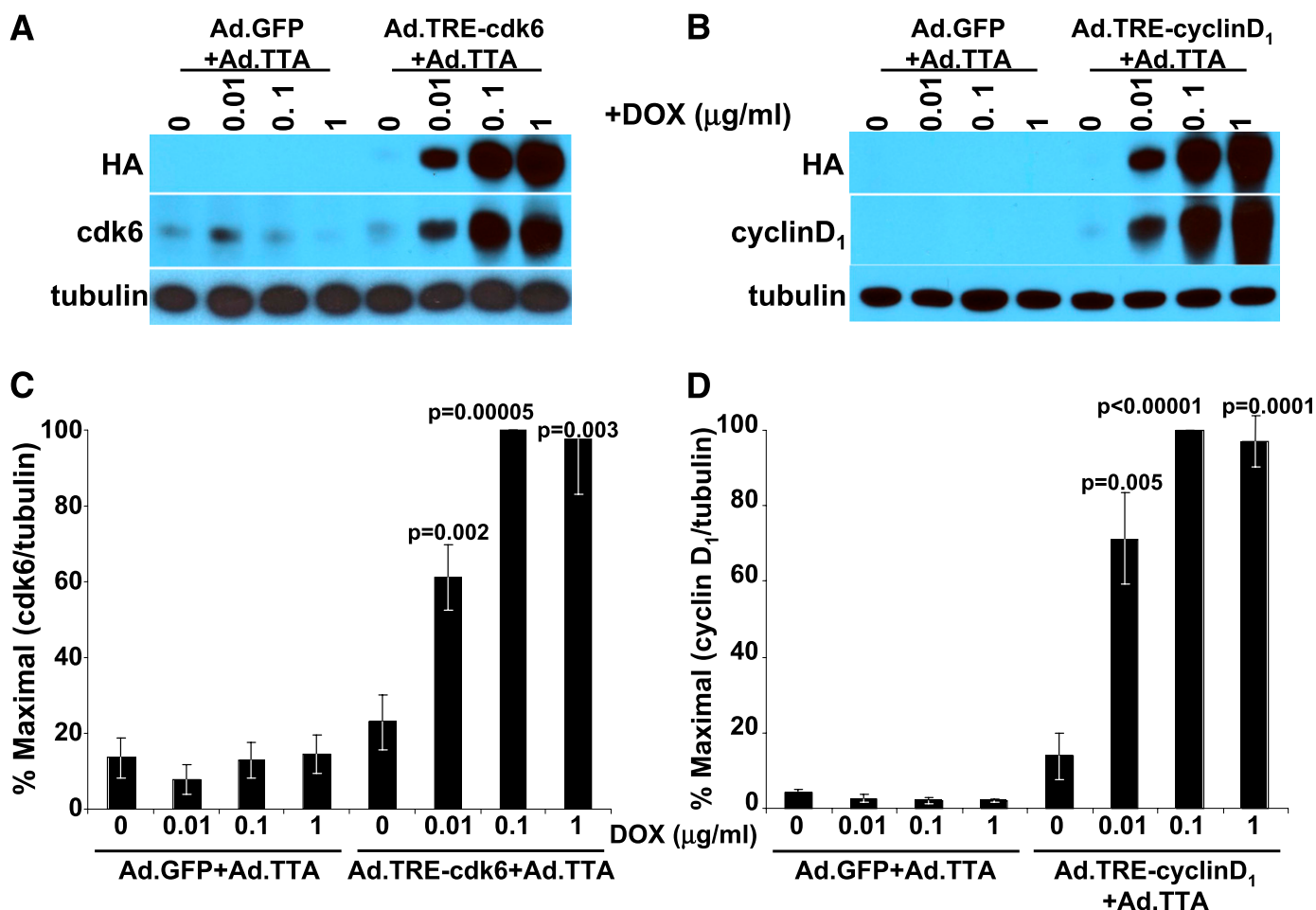


FIG. 1. Dose-related, Dox-induced expression of cdk6 or cyclin D₁ from Ad.TRE in human islets. *A* and *B*: Immunoblots of induced cdk6 or cyclin D₁ expression, respectively, in intact human islets 72 h posttransduction with adenoviruses as indicated, and treated with or without increasing doses of Dox. Delivery of cdk6 and cyclin D₁ was confirmed using the HA epitope tag. *C* and *D*: Densitometric quantification of *A* and *B* ($n = 5$). Values are expressed as a percentage of the Dox 0.1 group. *P* values refer to significance of indicated bars as compared with Ad.GFP-Ad.TTA controls as determined by Student unpaired *t* test. Error bars indicate SEM. Human islet donor information was as follows: mean age 43.7 ± 2.1 years; gender 16 men, 17 women; BMI 27.5 ± 0.9 ; purity $68.7 \pm 3.5\%$. Islets were maintained in RPMI 1640 medium containing 5.5 mmol/L glucose, 10% tet-free FBS (Clontech, Mountain View, CA), and 1% penicillin/streptomycin, unless otherwise stated. Two types of adenoviruses were used in this tet-on system. In the first, the TTA is driven by the CMV promoter. In the second, the cDNAs encoding either human cyclin D₁ or human cdk6 were controlled by the TRE. Both the cdk6 and cyclin D₁ constructs contained a HA epitope tag. The adenoviruses were generated using pJMI7 (8,9) and a modified pAC-CMVpLpA (8,9). In the Ad.TRE-ckd6 and Ad.TRE-cyclin D₁ viruses, the CMV promoter in the pAC-CMVpLpA plasmid was replaced with the MfeI-EcoRI promoter fragment of the pTRE-Shuttle2 from the Tet-On System (Clontech), now referred to as pAC-TRE, and the cdk6-HA and HA-cyclin D₁ cDNAs were ligated into pAC-TRE. Similarly, the cDNA for the reverse TTA from pTet-On Advanced (Clontech) was subcloned into pACCMVpLpA to produce the TTA adenovirus. Intact islets were transduced with adenovirus at a total of 250 multiplicities of infection (MOI) (150 MOI of Ad.TRE/100 MOI of Ad.TTA) in RPMI 1640 medium for 1 h. In some experiments, islets were dispersed by trypsinizing for 10 min at 37°C. Complete medium was added to stop the digestion. These single dispersed human islet cells were plated on 12-mm glass coverslips in 24-well plates and transduced at 150 MOI (50 MOI of each virus) in complete media for 2 h. Dox was added to the media immediately after transduction and replaced every other day as depicted. (A high-quality color representation of this figure is available in the online issue.)

maximal amount of cdk6 expressed by Ad.TRE-ckd6 (with 0.1 µg/mL Dox), whereas Ad.TRE-ckd6 without Dox exposure expressed 23% of the maximum; similarly, Ad.GFP-transduced islets and Ad.TRE-cyclin D₁ without Dox expressed 2.7 and 13.9% of maximal cyclin D₁ levels, respectively. This suggests some "leakiness" of the TRE promoter, but below levels associated with activation of proliferation (see below).

To determine the optimal time course for induction, human islets were transduced, placed in media containing 1 µg/mL Dox, and then harvested 24, 48, or 72 h later. Supplementary Fig. 1 demonstrates that both cell cycle proteins increase rapidly over 72 h. There was no further increase after 72 h (not shown).

Induction of human β-cell proliferation by Dox. Using laser confocal microscopy on isolated dispersed islets on coverslips, we examined BrdU incorporation into insulin⁺

cells. In Fig. 2A, negative controls (Ad.TRE-ckd6 and Ad. RE-cyclin D₁ without Dox, or Ad.GFP with Dox) did not show adenovirus-derived expression of cdk6 or cyclin D₁ (no white hemagglutinin [HA] epitope tag staining). On the other hand, cdk6/cyclin D₁ staining (white HA tag) was clearly induced by Dox in the right panels. Importantly, it is also clear that the adenoviruses were expressed in β-cells, because the white HA tag colocalizes in many green insulin⁺ cells. Most importantly, BrdU staining can be seen prominently in human β-cells expressing cdk6/cyclin D₁. In the bottom right panels, the HA staining has been separated from the merged images to more clearly demonstrate the BrdU staining in insulin⁺ cells.

These experiments were repeated using 13 different human islet preparations and quantified, as shown in Fig. 2B.

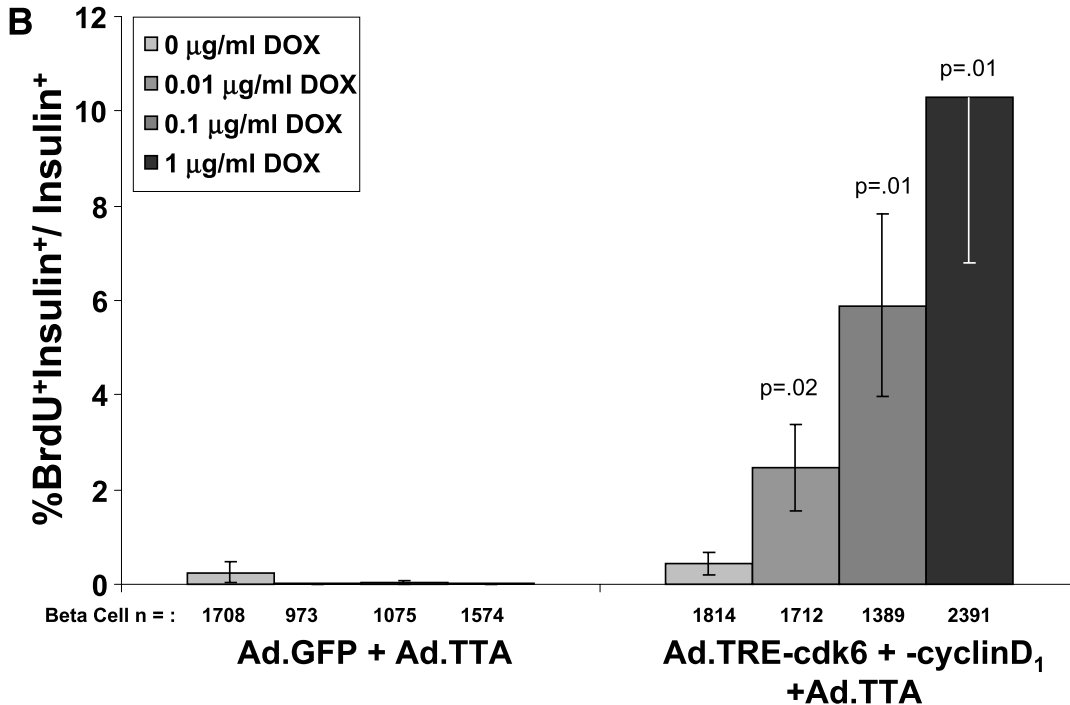
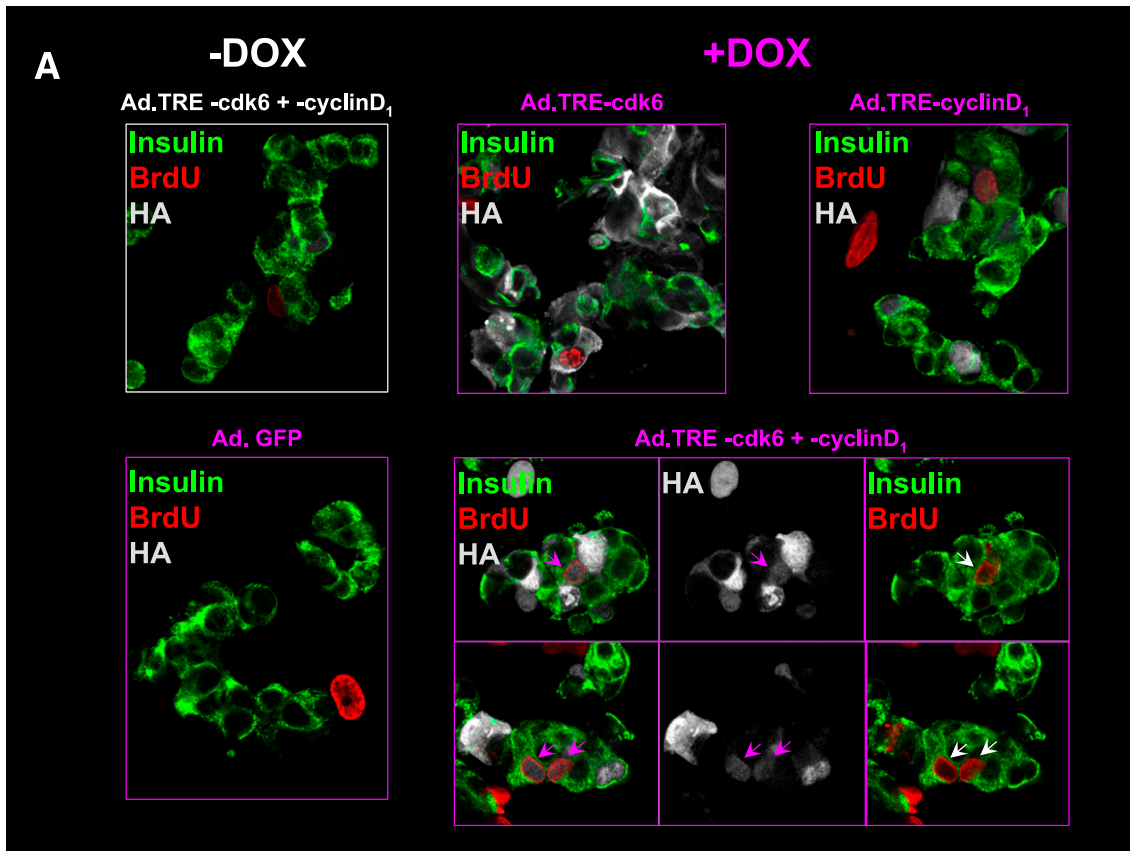


FIG. 2. Proliferation in human islets transduced with cdk6 and cyclin D₁ adenoviruses. **A:** Dispersed human islets were plated and transduced with the indicated adenoviruses (in combination with Ad.TTA) without Dox in the media (white border) or with 1 µg/mL Dox (pink border). After 3 days in culture, BrdU was added to the medium and the cells were fixed 18 h later. The dispersed islets were immunostained and visualized using laser confocal microscopy for insulin, BrdU, and HA (which is indicative of either adenovirally derived cdk6 or cyclin D₁, both of which contain an HA epitope tag). Arrows in the bottom right panels indicate BrdU-positive β -cells. Magnification is at 40x. **B:** Quantification of immunohistochemistry in **A** ($n = 13$). The numbers under each bar refer to the numbers of β -cells counted per condition. *P* values refer to significance of indicated bars as compared with their Ad.GFP/Ad.TTA controls using Student unpaired *t* test. Error bars indicate SEM. BrdU (1:1,000 dilution) (GE Healthcare, Pittsburgh, PA) was added to the islet media 18 h before fixation in 4% paraformaldehyde in phosphate-buffered saline for 10 min at room temperature. Cells were immunostained with anti-BrdU antibody 1:500 (Abcam, Cambridge, MA), anti-insulin antibody 1:1,000 (DAKO,

As can be seen, although very few insulin⁺ cells were proliferating (BrdU⁺) when transduced either with the Ad.GFP or with the Ad.TTA+Ad.TRE-cyclin D₁/cdk6 in the absence of Dox, transduction of β -cells with Ad.TRE-cdk6/cyclin D₁ in the presence of increasing amounts of Dox led to marked, dose-related increases in β -cell BrdU incorporation, ranging from 2.5 up to 10%. Thus, it is possible to activate adult human β -cell proliferation by the introduction of Dox in a dose-dependent manner. Importantly, glucose-stimulated insulin secretion was not adversely affected by induction of proliferation, and insulin and pdx1 mRNA expression also were not diminished (Supplementary Fig. 2).

Expression of cdk6 and cyclin D₁ does not induce cell death of transduced human islets. Transferase-mediated dUTP nick-end labeling (TUNEL) staining of transduced dispersed islets performed after 3 days of cdk6/cyclin D₁ induction revealed no increase in the rare TUNEL-positive cells (Supplementary Fig. 3A and B). Cleaved caspase-9, a product of the intrinsic apoptotic pathway, was not significantly elevated in islets overexpressing cdk6 and cyclin D₁. Similarly, cleaved caspase-3, activated in both the extrinsic and intrinsic pathways, independently confirmed that there was no increase in cell death, despite activation of cell cycle proliferation by cdk6/cyclin D₁. Interleukin (IL)-1 β treatment (100 units/mL) had no effect on TUNEL immunostaining in Ad.cdk6/cyclin D₁-treated islets in the presence of Dox (mean \pm SEM, 0.3 \pm 0.31% of insulin-positive cells) vs. controls (Ad.GFP 1.4 \pm 0.8%, or Ad.cdk6/cyclin D₁ islets with no Dox 0.95 \pm 0.94; $P = \text{NS}$, one-way ANOVA). Similarly, cell death as assessed using cleaved caspase-3 and -9 did not increase in response to IL-1 β in cdk6/cyclin D₁-expressing islets (not shown).

Removal of Dox results in normalization of cdk6/cyclin D₁ expression and cessation of human islet proliferation. To mimic normal developmental events, and to avoid oncogenic concerns, we explored whether, and how quickly, the induced cdk6/cyclin D₁ might decline after Dox withdrawal. Six different human islet preparations were transduced with adenoviruses and incubated for 3 days in complete medium containing 1 $\mu\text{g/mL}$ Dox, at which time they were washed with phosphate-buffered saline and tet-free medium was added. Islets were then either harvested immediately (referred to as "day 0") or at day 7. Figure 3 shows that 1) in six human islet preps, cdk6 and cyclin D₁ return to baseline levels observed in control-transduced human islets; and 2) when administered in combination (as well as when expressed individually as in Supplementary Fig. 4), they both return to baseline within 7 days of Dox withdrawal. Supplementary Fig. 4 further indicates, using an additional six human islet preparations, that 1) the decline in human islets is progressive over the course of 1 week, declining by 82 and 94%, respectively; and 2) the decline in cdk6 and cyclin D₁ occurs with comparable timing whether administered in combination (as in Fig. 3) or individually (as in Supplementary Fig. 4).

We next queried whether it was possible to reverse the proliferative effect in human β -cells by withdrawing Dox. Figure 4A independently confirms results in Fig. 2, showing that expressing cdk6 and cyclin D₁ in β -cells for 3 days leads to an increase in BrdU incorporation (top row). It further demonstrates that this proliferation continues for at least 10 days in the presence of Dox (second row). Significantly, 3 days of Dox exposure followed by a 7-day washout completely abolished the proliferation (BrdU immunostaining) in β -cells (third row). Proliferation in β -cells promptly resumed when Dox was reintroduced (bottom row). In Fig. 4B, the number of BrdU⁺ and insulin⁺ cells over time is quantified, confirming that 3 days of Dox induces robust proliferation, which disappears within 7 days of Dox withdrawal, and can be reintroduced to the full prior extent by re-exposure to Dox for 3 days.

DISCUSSION

These studies converged on two principal goals. First, they sought to determine whether it is possible to reversibly activate, inactivate, and reactivate human β -cell replication. And second, they sought to do this in a way that mimics the temporal sequence of human fetal β -cell replication, with proliferation being activated for a period of a few weeks or months, with the β -cells then allowed to return to quiescence. The results make the novel points that it is indeed possible to inducibly and transiently activate adult human β -cell replication, to temporally mimic normal proliferative events in human fetal β -cells, and to do so in a way that might navigate away from oncogenic concerns, while also retaining β -cell function and survival.

We have shown that several members of the G1/S family of molecules, such as cdk6 and cyclin D₁, are able to effectively drive adult human β -cell cycle progression (8–11). Notably, we have also shown that when transplanted into immunodeficient diabetic mice, cdk6- and cyclin D₁-overexpressing human β -cells continue to proliferate for 6–8 weeks, and do so in a way that actually improves β -cell function/glycemic control (9,10). Although these prior studies are promising, they are also a cause for worry, because they used adenoviral transduction using the constitutive CMV promoter, which leads to sustained expression. This raises the realistic concern that sustained overexpression of cdk6 and cyclins, many of which are well-established oncogenes in other contexts, might lead to tumor formation in transplanted islets exposed to these agents.

The first current goal, developing a means of reversibly inducing, then reversing, then reactivating brisk human β -cell replication, was achieved. These findings make the point that it is indeed possible to reversibly activate as well as arrest human β -cell replication, events that provide hope that strategies to allay oncogenic concerns associated with sustained cell cycle activation may be feasible.

The second goal, mimicking the transient proliferation that occurs in normal human development, was also achieved. Dox was able to activate, its withdrawal inactivate, and its reintroduction reactivate adult human β -cell

Carpinteria, CA), anti-cdk6 antibody 1:100 (Santa Cruz, Santa Cruz, CA), anti-cyclin D₁ antibody 1:100, and anti-HA antibody 1:50. Staining was visualized using Alexa Fluor secondary antibodies (Invitrogen, Carlsbad, CA) on an Olympus Fluoview 1000 confocal microscope. Cell death was assessed by the DeadEnd Fluorometric TUNEL System (Promega, San Luis Obispo, CA) according to manufacturer's instructions. For the positive controls, cells were exposed to 27 units DNase-I for 10 min at room temperature or, in the case of the cytokine treatment, 200 units/mL IL-1 β , 1,000 units/mL TNF- α , and 1,000 units/mL IFN- γ for 24 h in serum-free medium (3). Only cells with BrdU-stained nuclei that were completely surrounded by insulin staining were counted as BrdU-positive β -cells. (A high-quality digital representation of this figure is available in the online issue.)

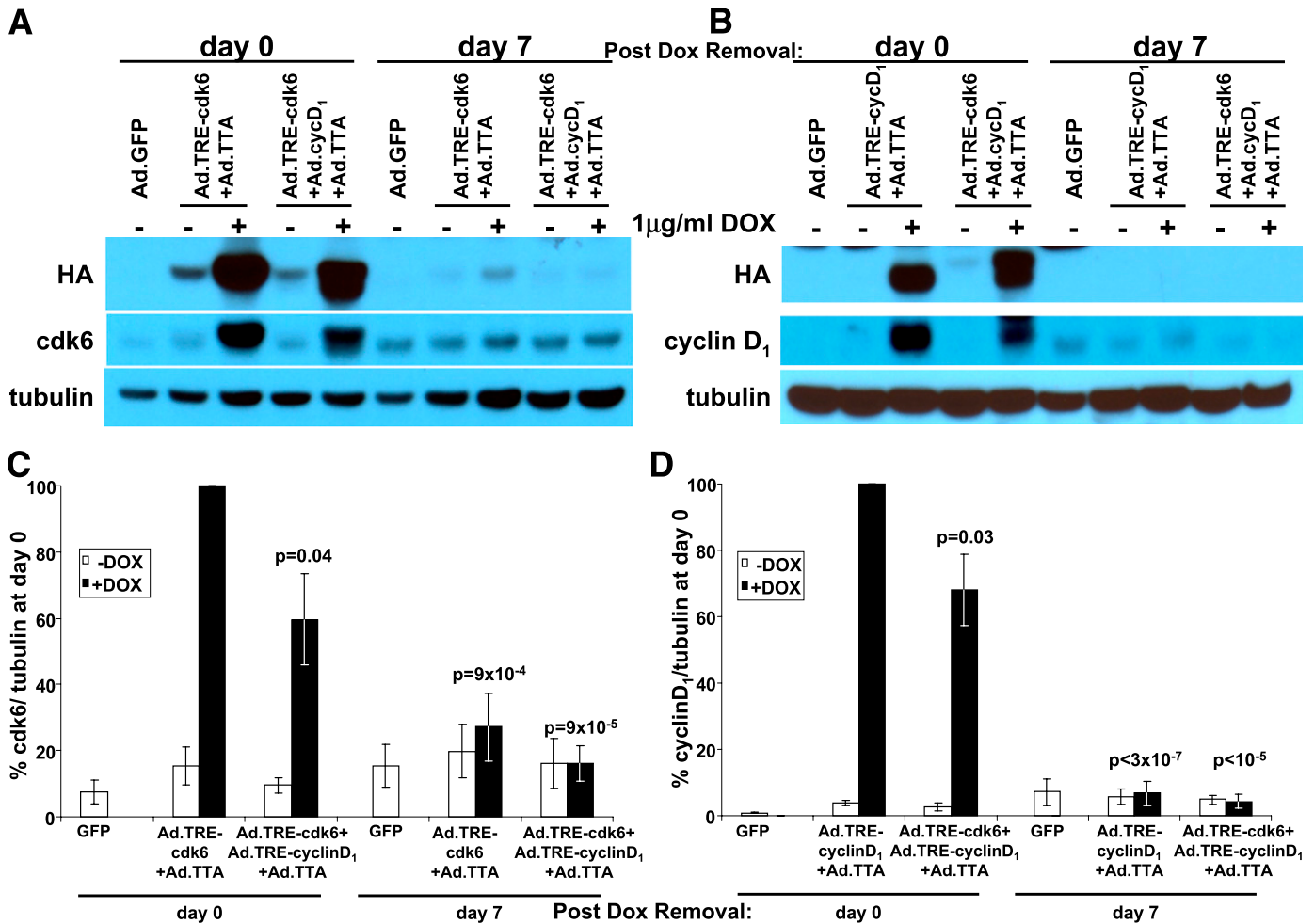


FIG. 3. Time-dependent reversal of cdk6 and cyclin D₁ expression. *A* and *B*: The reversal of cdk6 and cyclin D₁ overexpression, with return to endogenous levels observed in control human islets by day 7, as seen both by cdk6 and cyclin D₁ immunoblots as well as by immunoblots of the HA epitope tags. *C* and *D*: Quantified results of six separate experiments with human islets, demonstrating that after Dox withdrawal, cdk6 and cyclin D₁ expression are indistinguishable from that seen in control human islets. Also, note that in these experiments, in contrast to Supplementary Fig. 4 in which cdk6 and cyclin D₁ adenoviruses were administered separately, here both were administered simultaneously to each human islet preparation. Bars indicate mean \pm SEM. The decline in both cdk6 and cyclin D₁ was significant by one-way ANOVA ($P < 0.017$ for cdk6 and $P < 0.001$ for cyclin D₁). P values shown over the bars indicate differences between Dox-treated groups on day 7 versus day 0. There were no statistically significant differences between the groups on day 7. (A high-quality color representation of this figure is available in the online issue.)

replication. The time span we used (3, 7, and 10 days) was briefer than that which occurs in normal development (weeks to months), but further extending proliferation with longer exposure to Dox is clearly possible, because we have already demonstrated that prolonged (2 months) induction of human β -cell replication is possible using these approaches (9,10,15). The proliferation rates herein were comparable to rates achieved by constitutive CMV promoter adenoviruses in prior studies (9,10). Significantly, we were also able to achieve rates of proliferation that are comparable to, or exceed, those observed in developing human pancreas (12–14). These observations support the notion that induction of human β -cell replication at the 5% range for 2–3 months may be sufficient to expand endogenous and ex vivo sources of human β -cells.

These studies also have limitations. One is that they use gene therapy techniques, and as such are unlikely to be directly acceptable for human diabetes therapy. Hence, they serve as proof-of-principle studies, demonstrating that therapeutic, reversible human β -cell expansion is possible, and

provide additional impetus and targets for searches for small molecules, growth factors, nutrients, and signaling pathways that will drive expansion of adult human β -cells, whether they are derived from cadaveric or stem cell sources. Another limitation is that no in vivo transplant studies were performed. However, because those experiments are expensive and time consuming, and because we already have shown in two prior studies that transplant of human islets expressing cdk6 and cyclin D₁ function superiorly as compared with control human islets (9,10), the incremental knowledge gained through such experiments would be marginal.

In summary, these studies demonstrate that it is technically feasible to induce robust adult human β -cell replication in a regulated manner and to reverse this replication, attenuating oncogenic concerns and mimicking the transient β -cell proliferation that normally occurs in the developing human pancreas. These studies document that with the expanding list of β -cell cycle targets, and small molecule screens that are in progress, regulated proliferation of adult human β -cells is a feasible goal.

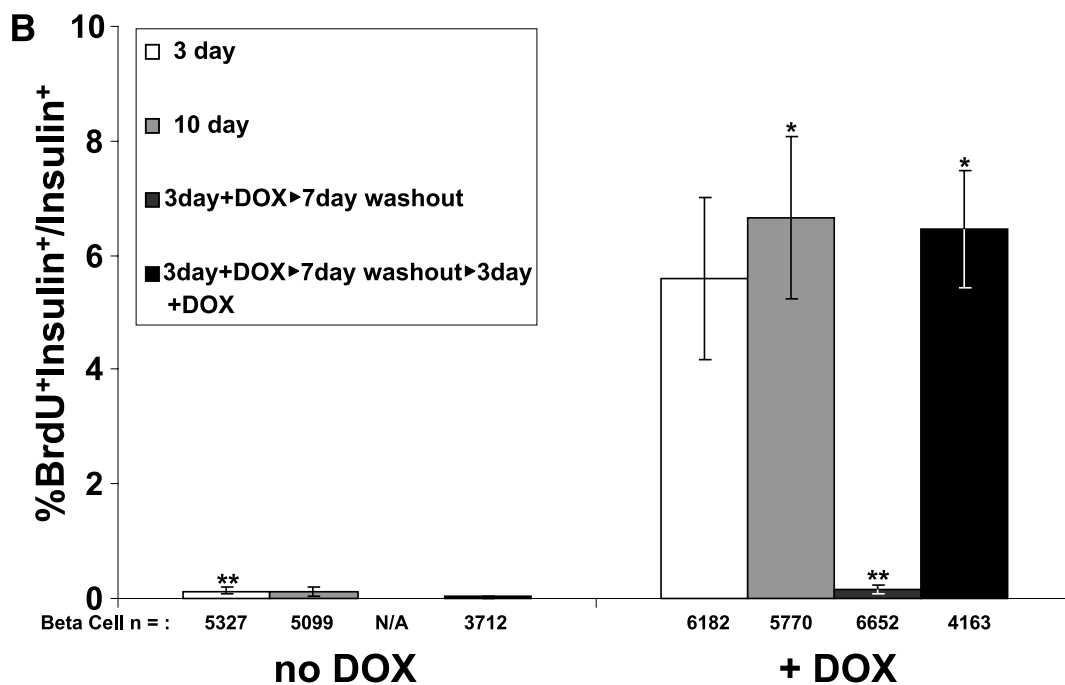
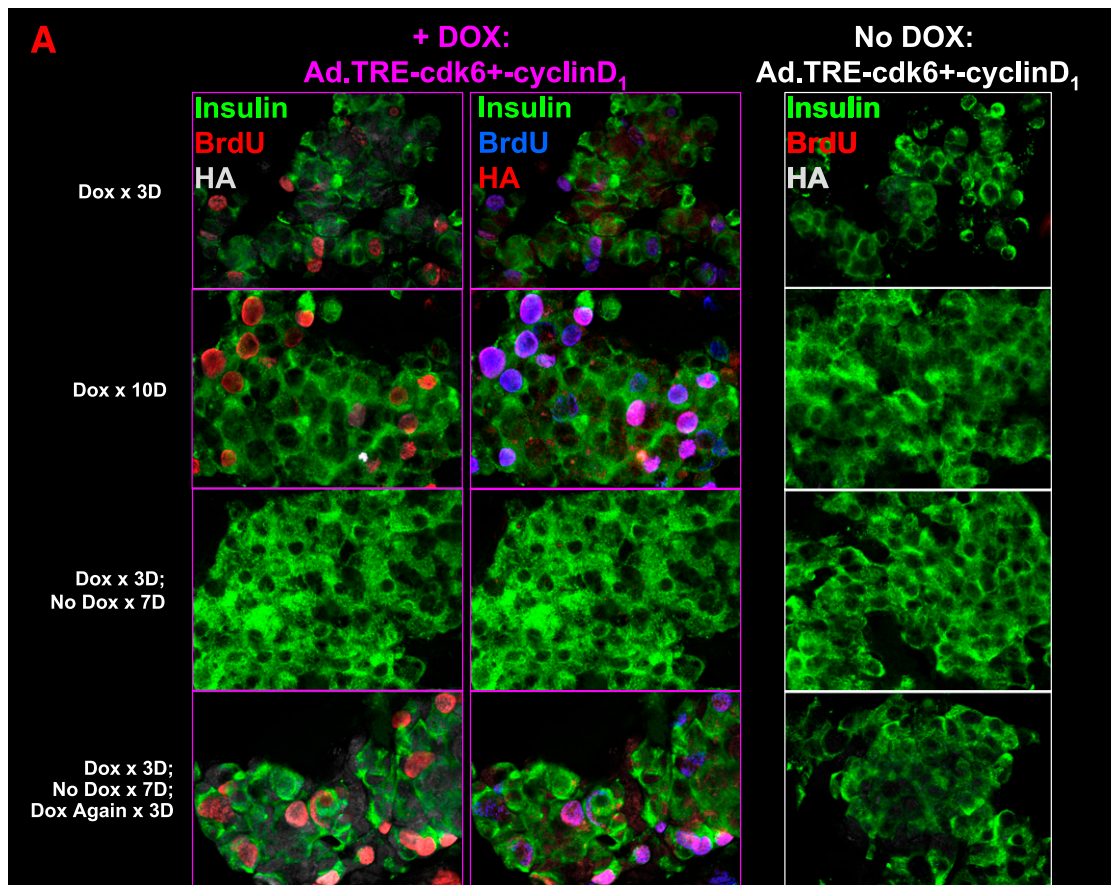


FIG. 4. Reversibility of proliferation in human islets transduced with cdk6 and cyclin D₁ adenovirus after Dox removal. **A:** Confocal microscopy of human islets (nine different preparations) dispersed to single cells, plated on coverslips, and transduced with the indicated adenoviruses without Dox (white boxes) in the media or with 1 μ g/mL Dox (pink boxes) for 3 (top row), 10 (second and third rows), or 13 days (bottom row). Islet cells were incubated with Dox for 3 days followed by a 7-day washout period (third row) and then re-exposure to Dox for an additional 3 days (bottom row). BrdU was added to the media 18 h before fixation. The dispersed islets were immunostained for insulin (green), BrdU (red), and adenovirally delivered cdk6 or cyclin D₁ (indicated by the white HA epitope tag). 40 \times magnification. **B:** Quantification of immunohistochemistry in **A**. The numbers under each bar refer to the numbers of β -cells counted per condition. Comparisons were made using unpaired Student *t* test (**P* < 0.05) or by one-way ANOVA with Tukey post hoc analysis (***P* < 0.05). Error bars indicate SEM. (A high-quality digital representation of this figure is available in the online issue.)

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K.K.T. researched data, contributed to discussion, and wrote the manuscript. J.W.K. and F.G.S. researched data. N.M.F.-T. contributed to discussion. A.F.S. originated the initial idea, contributed to discussion, and wrote the manuscript. K.K.T. and A.F.S. are guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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REFERENCES

- Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* 2003;52:102–110
- Perl S, Kushner JA, Buchholtz BA, Meeker AK, Stein GM, Hsieh M, Kirby M, Pechhold S, Liu EH, Harlan DM, Tisdale JF. Significant human β -cell turnover is limited to the first three decades of life as determined by in vivo thymidine analog incorporation and radiocarbon dating. *J Clin Endocrinol Metab* 2010;95:E234–E239
- Menge BA, Tannapfel A, Belyaev O, et al. Partial pancreatectomy in adult humans does not provoke beta-cell regeneration. *Diabetes* 2008;57:142–149
- Butler AE, Cao-Minh L, Galasso R, et al. Adaptive changes in pancreatic beta cell fractional area and beta cell turnover in human pregnancy. *Diabetologia* 2010;53:2167–2176
- Levitt HE, Cyphert TJ, Pascoe JL, et al. Glucose stimulates human beta cell replication in vivo in islets transplanted into NOD-severe combined immunodeficiency (SCID) mice. *Diabetologia* 2010;54:572–582
- Parnaud G, Bosco D, Berney T, et al. Proliferation of sorted human and rat beta cells. *Diabetologia* 2008;51:91–100
- Maedler K, Schumann DM, Schulthess F, et al. Aging correlates with decreased beta-cell proliferative capacity and enhanced sensitivity to apoptosis: a potential role for Fas and pancreatic duodenal homeobox-1. *Diabetes* 2006;55:2455–2462
- Cozar-Castellano I, Takane KK, Bottino R, Balamurugan AN, Stewart AF. Induction of beta-cell proliferation and retinoblastoma protein phosphorylation in rat and human islets using adenovirus-mediated transfer of cyclin-dependent kinase-4 and cyclin D1. *Diabetes* 2004;53:149–159
- Fiaschi-Taesch NM, Bigatel TA, Sicari BM, et al. Survey of the human pancreatic beta-cell G1/S proteome reveals a potential therapeutic role for cdk-6 and cyclin D1 in enhancing human beta-cell replication and function in vivo. *Diabetes* 2009;58:882–893
- Fiaschi-Taesch NM, Salim F, Kleinberger J, et al. Induction of human beta-cell proliferation and engraftment using a single G1/S regulatory molecule, cdk6. *Diabetes* 2010;59:1926–1936
- Guthalu Kondegowda N, Joshi-Gokhale S, Harb G, et al. Parathyroid hormone-related protein enhances human β -cell proliferation and function with associated induction of cyclin-dependent kinase 2 and cyclin E expression. *Diabetes* 2010;59:3131–3138
- Meier JJ, Butler AE, Saisho Y, et al. Beta-cell replication is the primary mechanism subserving the postnatal expansion of beta-cell mass in humans. *Diabetes* 2008;57:1584–1594
- Kassem SA, Ariel I, Thornton PS, Scheimberg I, Glaser B. Beta-cell proliferation and apoptosis in the developing normal human pancreas and in hyperinsulinism of infancy. *Diabetes* 2000;49:1325–1333
- Köhler CU, Olewinski M, Tannapfel A, Schmidt WE, Fritsch H, Meier JJ. Cell cycle control of β -cell replication in the prenatal and postnatal human pancreas. *Am J Physiol Endocrinol Metab* 2011;300:E221–E230
- Fiaschi-Taesch NM, Berman DM, Sicari BM, et al. Hepatocyte growth factor enhances engraftment and function of nonhuman primate islets. *Diabetes* 2008;57:2745–2754
- Rao P, Roccisana J, Takane KK, et al. Gene transfer of constitutively active Akt markedly improves human islet transplant outcomes in diabetic severe combined immunodeficient mice. *Diabetes* 2005;54:1664–1675