Differentiation of mouse nuclear transfer embryonic stem cells into functional pancreatic beta cells

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Abstract

Aims/hypothesis Therapeutic cloning has been reported to have potential in the treatment of several degenerative diseases. However, it has yet to be determined whether mouse nuclear transfer-embryonic stem cells (NT-ESCs) can be differentiated into pancreatic beta cells and used to reverse diabetes in an animal model.

Methods We first used the somatic nuclear transfer technique to generate mouse NT-ESCs and then developed a chemically defined stepwise protocol to direct the NT-ESCs into functional pancreatic beta cells. We examined the gene expression pattern of the differentiated NT-ESCs and transplanted the NT-ESC-derived insulin-producing cells into recipient diabetic mice.

Results Four mouse NT-ESC lines were first established using an improved nuclear transfer technique and insulin-

producing cells were efficiently generated from NT-ESCs by mimicking pancreatic *in vivo* development. Most of the insulin-producing cells that we generated co-produced pancreatic and duodenal homeobox 1, but not glucagon at the final stage of this differentiation method, which differed from the insulin and glucagon co-production reported by other groups. The differentiated NT-ESCs were able to release insulin in response to glucose stimuli and normalise the blood glucose level of diabetic mice for at least 2 months.

Conclusions/interpretation These results demonstrate the potential of therapeutic cloning for cell therapy of type 1 diabetes in a mouse model.

Keywords NT-ESC · Nuclear transfer-embryonic stem cell · Pancreatic beta cell differentiation · Therapeutic cloning

W. Jiang and Z. Bai contributed equally to this paper.

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Abbreviations

bFGF	basic fibroblast growth factor
EGF	epidermal growth factor
ESC	embryonic stem cell
FOXA2	forkhead box A2
ICM	inner cell mass
ITS	insulin–transferrin–selenium
NT	nuclear transfer
NT-ESC	nuclear transfer-embryonic stem cell
PDX1	pancreatic and duodenal homeobox 1
RA	<i>all-trans</i> retinoic acid
SOX	SRY (sex determining region Y)-box

Introduction

Therapeutic cloning, which utilises healthy cells from a patient to create genetically matched embryonic stem cells (ESCs) by nuclear transfer (NT) and then drives the NT-

ESCs to differentiate into specialised functional cells, offers an attractive potential in treatment of many degenerative diseases such as haemopoietic disease in mouse models [1]. However, it has not yet been reported whether NT-ESCs could be induced to differentiate into pancreatic beta cells and reverse diabetes in an animal model.

Type 1 diabetes results from loss of the pancreatic beta cells. At present, pancreatic islet transplantation has become a promising therapeutic option for patients with type 1 diabetes. However, this therapy is not widely used because of the severe shortage of donor islets and the problem of severe immune rejection. We hypothesised that a combination of the NT-ESC and differentiation techniques could be used to generate customised ESCs and to direct differentiation of the NT-ESCs into pancreatic beta cells as a promising resource for cell therapy to treat diabetes without requiring the use of immune-suppressor drugs.

Previously, ESCs have been shown to differentiate into pancreatic insulin-producing cells by our laboratory and other groups [2–6]. In our previous study, we established a stepwise induction method to differentiate mouse ESCs into functional pancreatic beta cells by treatment with a combination of activin A, all-*trans* retinoic acid (RA) and other maturation factors, and the differentiated pancreatic beta cells proved able to cure diabetic mice [3]. Recently, we adapted the protocol for human ESCs and successfully differentiated human ESCs into functional insulin-producing cells [5]. Several other groups have also developed protocols to differentiate human ESCs into insulin-producing cells in line with pancreatic development [4, 6]. However, the reported efficiency is still very low in general, one of the major problems being that glucagon was co-produced with insulin in the differentiated cells [4, 6].

Here, we have successfully established mouse ESC lines by an improved NT technique and developed a chemically defined stepwise method to direct the NT-ESCs into functional pancreatic beta cells. Insulin-producing cells were effectively generated, which did not co-produce glucagon. The differentiated NT-ESCs expressed many pancreatic beta cell markers and secreted insulin in response to glucose and KCl stimulation. Furthermore, the NT-ESC-derived insulin-producing cells were transplanted into recipient diabetic mice and reversed hyperglycaemia.

Methods

Creation of cloned embryos The Institutional Animal Care and Use Committee of Peking University approved all animal procedures. Mouse fibroblast cells from the fetus F1 (C57BL/6×DBA) at 16.5 days post-coitum were cultured in DMEM (Gibco-BRL, Rockville, MD, USA) containing 20% FCS (vol./vol.; Gibco) until 100% confluency and

then exposed to DMEM supplemented with 0.5% FCS for 3 days for further NT as described previously [7]. The cloned embryos were cultured in KSOM medium (a potassium simplex optimised medium) [8] until the four- to eight-cell stage and subsequently treated by 0.05 µg/ml insulin for additional 3 days to the hatched blastocyst stage.

Establishment and characterisation of the NT-ESC lines For the establishment of the NT-ESC line, the cloned blastocysts were transferred into knockout DMEM (Gibco) with 20% knockout serum replacement (vol./vol.; Gibco) containing 1,000 units/ml of leukemia inhibitory factor (Gibco) on a feeder layer treated with mitomycin-C (Roche, Indianapolis, IN). Following culture on the feeder for 13 to 14 days, the outgrowth was dissociated by needles and passaged for two times, then digested with trypsin to passage.

For teratoma assay, approximately 1×10^6 NT-ESCs were injected into the peritoneal of the donor mice and about 6 weeks later the injected mice were examined. The teratomas were embedded in paraffin and sectioned for histological analysis by haematoxylin–eosin staining. Additionally, a tetraploid blastocyst injection was performed to obtain cloned mice. About eight NT-ESCs were injected into the tetraploid blastocyst, assisted by a brief pulse of piezo micromanipulator, and the injected blastocysts were transferred into one uterine horn of pseudo-pregnant female mice at 2.5 days post coitum.

Differentiation of the NT-ESCs into pancreatic beta cells The protocol for differentiation of NT-ESCs into beta cells was modified and improved according to our previous reports [3, 5] and performed in a serum-free and chemically defined system. First, NT-ESCs were plated into Matrigel-coated (1:50; BD Biosciences, BD Biosciences, Bedford, MA, USA) Petri dishes (Nunc, Roskilde, Denmark) and incubated for 3 days with X-Vivo 10 (Cambrex, East Rutherford, NJ, USA) supplemented with 1 g/l BSA (Sigma, St Louis, MO) containing 50 ng/ml activin A (Sigma). Then the differentiated cells were cultured for 2 days in F12/Iscove's modified Dulbecco's medium (1:1) supplemented with 5 g/l BSA and 1% insulin–transferrin–selenium (ITS, by vol.; all from Gibco) with 2 µmol/l RA (Sigma). After RA induction, the cells were cultured in DMEM (low glucose; Gibco) supplemented with 5 g/l BSA and 1% ITS, together with 10 ng/ml basic fibroblast growth factor (bFGF; Sigma) and 20 ng/ml epidermal growth factor (EGF; Sigma) for 3 days and another 3 to 5 days in DF12 (Gibco) medium with ITS, 10 ng/ml bFGF and 10 mmol/l nicotinamide (Sigma) for maturation.

Immunofluorescence assay The NT-ESCs or induced cells were fixed in 4% paraformaldehyde (wt/vol.) and blocked with 10% serum and 0.2% Triton X-100 (vol./vol.) in PBS,

then incubated overnight at 4°C with primary antibody (Electronic supplementary material [ESM] Table 1) and further incubated with secondary antibody (fluorescein isothiocyanate (FITC) or tetramethylrhodamine B isothiocyanate (TRITC)-conjugated donkey anti-rabbit or anti-goat or anti-mouse IgG; Jackson Laboratories, West Grove, PA, USA). Images were captured using a phase contrast fluorescent microscope (IX-71; Olympus, Tokyo, Japan) or confocal microscope (TCS-SP2; Leica, Allendale, NJ, USA). Alkaline phosphatase activity was measured according to instructions of the manufacturer (Promega, Madison, WI, USA). The DeadEnd Fluorimetric TUNEL System (Promega) was used to perform the cell apoptosis assay.

The percentage of cells positive for pancreatic and duodenal homeobox 1 (PDX1) and C-peptide was calculated using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD, USA) as follows: an area to be measured was outlined by a tracer, with the number of counting frames preset so that the device sampled the measuring sites randomly. The software counted the value of the respective chosen areas. We used DAPI staining to estimate the amount of whole cells. To count the percentage of cells positive for PDX1 or C-peptide, the positive areas were estimated by the software in the same way. The percentage was calculated by the following formula: area positive for C-peptide (or PDX1)/whole DAPI-positive area. Pictures were taken from at least three independent cultures and 50 random and different views of the differentiated cells to estimate the positive cell percentage.

RT-PCR analysis of gene expression Total RNA was extracted by TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA was digested by RNase-free DNase I (Ambion, Austin, TX, USA) and then reverse-transcribed into cDNA by Moloney murine leukemia virus reverse transcriptase (Promega). PCR was performed with Ex Taq polymerase (TaKaRa, Otsu, Shiga, Japan) following the manufacturer's protocol. The cycle conditions were as follows: 94°C for 5 min, followed by 35 cycles (94°C denaturation for 30 s, 56–58°C annealing for 30 s, 72°C elongation for 35 s), with a final incubation at 72°C for 6 min.

Real-time PCR analysis was performed on a sequence detection system (ABI PRISM 7300; Applied Biosystem, Foster City, CA, USA) using the SYBR Green PCR Master Mix (Toyobo, Osaka, Japan). The PCR reaction consisted of 12.5 µl of SYBR Green PCR Master Mix, 0.4 µmol/l of forward and reverse primers, 10.5 µl water, and 1 µl template cDNA in a total volume of 25 µl. The relative expression of each gene was normalised against *Gapd* (also known as *Gapdh*). The primers sequences and the length of the product are shown in ESM Table 2.

Insulin release assay by ELISA The insulin levels in culture supernatants were measured by a rat/mouse insulin ELISA kit (EZRMI-13K; Linco, St Charles, MO, USA). After pre-incubation with Krebs–Ringer buffer at 37°C for 90 min, the differentiated cells were incubated at 37°C for 60 min with Krebs–Ringer buffer containing 2.5 mmol/l glucose or 27.5 mmol/l glucose or 30 mmol/l KCl. Then the respective conditioned mediums were collected and tested. The total protein content was detected with a protein assay kit (BCA; Pierce, Rockford, IL, USA).

Transplantation of insulin-producing cells under renal subcapsule Animals of the same strain of F1 male mice were injected with streptozotocin (Sigma; i.p., 180 mg/kg) at about 8 weeks of age and used as experimental diabetes model. When the blood glucose level of streptozotocin-treated mice was constantly above 13.9 mmol/l, about 1×10^6 differentiated cells were transplanted into the left renal capsule. PBS-treated and the spontaneously differentiated NT-ESCs without factors treatment were used as control. Cryostat sections of the operated kidneys were prepared and C-peptide levels in transplanted cells in the renal capsule were tested by immunofluorescence.

Results

Establishment and characterisation of NT-ESC lines We synchronised the donor cells by combined contact inhibition and serum starvation treatment and obtained a high ratio of

Table 1 Effect of contact inhibition and/or serum starvation on in vitro development of mouse cloned embryos

Parameter	Oocytes (n)	Fused, n (%)	Cleaved, n (%)	Blastocysts, n (%)
Contact inhibition	319	281 (88.1)	135 (48.0) ^a	49 (36.3)
Serum starvation	267	232 (86.9)	141 (60.8) ^b	58 (41.1)
Contact inhibition + serum starvation	395	340 (86.1)	236 (69.4)	95 (40.3)

Following combined contact inhibition and serum starvation treatment, the cleavage rate of the cloned embryos was significantly higher than that following serum starvation ($p < 0.05$) or contact inhibition treatment ($p < 0.01$), respectively. However, the blastocyst formation rate from the two-cell stage did not differ among the three treatment groups

^a $p < 0.01$ vs serum starvation group (cleaved); ^b $p < 0.005$ vs contact inhibition + serum starvation (cleaved)

Table 2 Effect of insulin on the in vitro development of cloned embryos

Medium	Fused oocytes (<i>n</i>)	Cleaved, <i>n</i> (%)	Blastocysts, <i>n</i> (%)	Blastocysts tested, <i>n</i>	Total cell number	ICM cell number
K-KII	92	57 (62.0)	28 (49.1)	15	63.2±15.6 ^a	17.1±5.7 ^a
K-KII + insulin	103	67 (65.0)	35 (52.2)	22	89.0±13.1	28.0±7.0

Values are means±SEM, unless otherwise stated

When cloned embryos were transferred into the KSOM-II medium supplemented with 0.05 µg/ml insulin at the four to eight cell stage and cultured to blastocyst stage, the cell number within the cloned ICM was significantly enhanced ($p<0.05$) compared with that of the control group, although the blastocyst formation rate ($p>0.05$) did not improve

^a $p<0.05$ for difference vs other value for K-KII + insulin in same column

K, KSOM; KII, KSOM supplemented with 0.5% essential amino acids and 0.5% non-essential amino acids (vol./vol.), and with concentration of glucose increased to 5.56 mmol/l

cloned cleavage embryos (Table 1). Then the cloned four to eight cell stage embryos were transferred into the medium supplemented with 0.05 µg/ml insulin to significantly enhance the cell number within the cloned inner cell mass (ICM) of the blastocyst (Table 2). Following nine cloned blastocysts transferred into the ESC media, four ESC lines (44.4%) were established. The resulting clone-derived ESCs had a high ratio of nucleus to cytoplasm as well as prominent nucleoli, and their colony morphology was similar to that of typical mouse ESCs (Fig. 1a).

The mouse NT-ESCs expressed specific pluripotent markers, including POU domain, class 5, transcription factor (POU5F1), Nanog homeobox (NANOG), SRY-box containing gene 2 (SOX2), stage-specific embryonic antigen 1 (SSEA1) and alkaline phosphatase (Fig. 1b–f, respectively). The ESCs had a normal karyotype (normal fraction >80%) (ESM Fig. 1a). Teratomas formed (ESM Fig. 1b) 6 weeks after the NT-ESCs injection and histological examination revealed that all three germ layers were generated: endoderm-derived gut epithelium (ESM Fig. 1c), ectoderm-derived neurons (ESM Fig. 1d), and

mesoderm-derived cartilage (ESM Fig. 1e) and adipose tissue (ESM Fig. 1f). Furthermore, four NT-ESC-tetraploid embryos developed into adults by tetraploid blastocyst injection (Fig. 1g) and delivered normal offspring (Fig. 1h) after natural mating.

Differentiation of NT-ESCs into beta cells in vitro We developed an efficient chemically defined protocol to differentiate the mouse NT-ESCs into functional pancreatic beta cells (Fig. 2a). Many T-positive cells were observed after 2 days with activin A treatment (Fig. 2b) and SOX17- and forkhead box A2 (FOXA2)-positive cells were also observed after 3 days with activin A treatment (Fig. 2c), suggesting that the NT-ESCs differentiated into mesendoderm then further into definitive endoderm cells. After RA treatment, PDX1-positive cells appeared (Fig. 2d). In the latter stage, the differentiated cells produced pancreatic endocrinal markers, including NEUROG3, NKX6-1, SOX9, PAX6 and ISL1 (Fig. 2e). These data suggested that the differentiation progress mimicked the in vivo pancreatic development to a certain extent.

Fig. 1 Characterisation of the NT-ESCs. **a** Phase contrast of NT-ESCs growing on top of feeder layer. **b–e** Presence of POU5F1 (**b**), NANOG (**c**), SOX2 (**d**) and SSEA1 (**e**); scale bar, 40 µm. **f** Alkaline phosphatase staining of NT-ESCs. **g** Adult NT-ESC-tetraploid mice

derived from NT-ESCs complemented by tetraploid embryos and **h** living offspring generated from the adult NT-ESC-tetraploid mice following natural mating

In the final stage, visible clusters formed that were C-peptide-positive and PDX1-positive as examined by immunofluorescence (Fig. 3a). Many NKX6-1- and KCNJ11 (potassium inwardly-rectifying channel, subfamily J, member 11)-positive cells were also observed (Fig. 4a,b). RT-PCR data showed that the differentiated cells expressed pancreatic beta cell-specific genes, including insulin I, insulin II, *Pdx1*, *Iapp*, *Isl1*, *Abcc8* and *Nkx2-2*, and other endocrinal markers, including somatostatin and glucagon (Fig. 3c). We observed about 5 to 10% PDX1- and C-peptide-positive cells in the final stage (data not show). To further confirm that the clusters contained mature beta cells, we performed co-staining of the C-peptide with PDX1 or glucagon. We found

that most of the clusters of C-peptide-positive cells were PDX1-positive (Fig. 3a), but glucagon-negative (Fig. 3b). These data are consistent with the known gene expression pattern of mature islet cells.

To determine whether insulin secretion from those differentiated NT-ESCs could be regulated by glucose or other physiological stimuli, we treated these cells with a high concentration of glucose or KCl and then analysed the insulin release level in culture medium by ELISA. We found that the insulin release with high glucose stimulation was significantly higher than that with low glucose (1.15 ± 0.26 vs 0.53 ± 0.04 pmol/mg protein, $p=0.005$) and that the differentiated cells could also respond to KCl stimulation

Fig. 2 Characterisation of the differentiation process. **a** Flow chart of the differentiation protocol. **b** t-complex (T) produced at day 2; **c** SRY-box containing gene 17 (SOX17) and FOXA2 produced at day 3; **d** PDX1 produced at day 5; and **e** neurogenin 3 (NEUROG3), NK6 transcription factor related, locus 1 (*Drosophila*) (NKX6-1), SRY-box containing gene 9 (SOX9), paired box gene 6 (PAX6) and ISL1 transcription factor, LIM/homeodomain (ISL1) produced at day 8. IMDM, Iscove's modified Dulbecco's medium. Scale bar, 100 μ m *T* brachyury homologue

Fig. 3 Differentiation of NT-ESCs to functional pancreatic cells. **a** C-peptide- and PDX1-positive clusters derived from NT-ESCs, with positive control for C-peptide from cryostat section of mouse pancreas, as indicated. **b** Distribution of C-peptide and glucagon at the final stage, with positive control for glucagon from cryostat section of mouse pancreas, as indicated. Scale bar (**a**, **b**), 100 μ m. **c** RT-PCR data of the differentiated NT-ESCs at the final stage. Lanes 1 and 2 represent two different experimental groups. Mouse islets were used as the positive

controls. **d** Analysis of insulin release of induced cells treated with high and low concentration of glucose and KCl. $p=0.005$ by Student's *t* test for 2.5 mmol/l vs 27.5 mmol/l; $p=0.001$ by Student's *t* test for 2.5 mmol/l vs 30 mmol/l. **e** The blood glucose of operated mice at different time points. Black diamonds, induced cells; white squares, PBS; white triangles, control cells (differentiated without factors treatment) **f** C-peptide staining in the grafted kidney of induced cell-transplanted mice (left) and non-induced cell-transplanted mice (right)

(1.20 ± 0.22 vs 0.53 ± 0.04 pmol/mg protein, $p=0.001$). These data therefore indicated that the differentiated NT-ESCs secreted insulin in response to glucose and KCl stimulation (Fig. 3d). Considering that the medium contained insulin and apoptotic cells could take up the insulin, we performed the TUNEL assay and observed that

PDX1-positive cells and C-peptide-positive cells were TUNEL-negative (Fig. 4c).

Transplantation of NT-ESC-derived insulin-producing cells reversed diabetes in diabetic mice After transplantation of NT-ESC-derived insulin-producing cells, the blood glucose of

Fig. 4 Characterisation of the differentiation cell at the final stage. NK6 transcription factor related, locus 1 (*Drosophila*; NKX6-1) (**a**) and potassium inwardly rectifying channel, subfamily J, member 11 (KCNJ11) (**b**) were stained at the final differentiated NT-ESC-derived cells. **c** The co-stain of TUNEL and PDX1 or C-peptide of the final differentiated NT-ESC-derived cells, with positive control of TUNEL by DNase treatment as indicated. **d** The co-stain of beta-III tubulin and C-peptide of the final differentiated NT-ESC-derived cells, with positive control of beta-III tubulin, as indicated. Scale bar, 100 μ m

cell-treated mice ($n=5$) was significantly reduced. In contrast, the blood glucose of the sham operated control (PBS-transplanted) mice ($n=7$) and the control cell-transplanted (without factors treatment group) mice ($n=4$) remained above 20 mmol/l. In addition, after removal of the left kidney transplanted with NT-ESC-derived beta cells, the blood glucose level reverted back to levels over 13.9 mmol/l (Fig. 3e).

C-peptide-positive cells were visualised in the cryostat sections of NT-ESC-derived beta cell-treated kidneys but not in the control cell-transplanted kidneys (Fig. 3f). These results indicate that the NT-ESC-derived insulin-producing cells improved glucose regulation and reversed the streptozotocin-induced diabetes in mice when transplanted into the renal capsule.

Discussion

The present study demonstrated the potential of therapeutic cloning in type 1 diabetes cell therapy. We successfully

established four mouse ESC lines by modified NT methods and differentiated the NT-ESCs into functional insulin-producing cells that were able to reverse streptozotocin-induced diabetes in mice.

A new and efficient chemically defined stepwise protocol for the differentiation of mouse NT-ESCs into insulin-producing cells was designed. Previously, we had developed a novel three-step protocol combined with activin A and RA to induce mouse ESC differentiation into pancreatic beta cells [3]. This was the first study demonstrating that the combination of activin A and RA could direct pancreatic islet fate from ESCs. The previous protocol was then successfully adapted for human ESC differentiation in a chemically defined system [5]. However, our previously established mouse protocol [3] involved spontaneous embryonic body formation and used fetal bovine serum, which could interfere with differentiation efficiency and reproducibility. In the present work, we developed a chemically defined protocol for cloned mouse ESCs that does not use embryonic body system. Furthermore, we found that EGF treatment could improve the

Fig. 5 Analysis of the effect of EGF treatment. **a** Immunostaining indicated that the neurogenin 3 (NEUROG3)-positive cells (at day 8) and PDX1-positive cells (at day 11) were significantly increased after EGF treatment. Scale bar, 100 μ m. **b** The related *Iapp* and insulin I mRNA expression at the final stage after EGF treatment. The gene expression of groups without EGF treatment was normalised to 100%. Black bars, EGF treatment; white bars, no EGF treatment

production of NEUROG3 and in the latter stage increased the expression of pancreatic beta cell marker genes, including *Pdx1*, *Nkx6-1* and *Iapp* (Fig. 5). Recently, Rolletschek et al. reported that our previous mouse protocol could lead ESCs into both pancreatic and neuronal differentiation [9]; however, we did not detect expression of neural marker beta-III tubulin in the C-peptide-positive cells at the final differentiation stage in our newly improved system (Fig. 4d).

Recently, several groups have reported protocols for the differentiation of insulin-positive cells from human ESCs [4, 6]. However, insulin and glucagon double-positive cells were observed at the final differentiation stage. In pancreatic development, the mutually exclusive expression of endocrinal insulin and glucagon in the mature islet is

crucial, because the doubly positive cells observed in the embryo pancreas have been shown by lineage tracing analysis to never give rise to adult endocrine cells as reviewed [10]. In our system, presence of glucagon in the C-peptide-positive clusters at the final differentiation stage was not detected (Fig. 3b), which suggested that the insulin-producing cells obtained by the present protocol were comparatively more mature.

Compared with freshly isolated mouse islets, the differentiated NT-ESCs revealed a quite lower level of insulin secretion (0.53 ± 0.04 vs 9.3 ± 3.2 pmol/mg at 2.5 mmol/l glucose; 1.15 ± 0.26 vs 39.3 ± 5.4 pmol/mg at 27.5 mmol/l glucose). However, considering only 5 to 10% final differentiated cells were C-peptide-positive, our results were reasonable. In the future, we will establish a method to purify the beta cell from the differentiated cells and further evaluate the physiological function of ESC-derived beta cells.

In the therapeutic cloning approach, the low developmental potential of cloned embryos needs to be improved to yield a high efficiency for derived NT-ESCs. We obtained a high ratio of cloned cleavage embryos and successfully established four ESC lines from nine cloned blastocysts (44.4%) in the present study. To acquire better NT efficiency, donor cells are generally subjected to a period of contact inhibition or serum starvation [11] for synchronisation at the G0/G1 phase. In our experiment, we combined the contact inhibition and serum starvation treatment to synchronise the donors and obtained improved cleavage of cloned embryos. It should also be noted that we found that insulin promoted proliferation of the ICM in cloned embryos, which is consistent with reported work on normal embryos [12].

In summary, the present study demonstrates that mouse NT-ESCs can differentiate into functional pancreatic beta cells and reverse diabetes in mice, illustrating the potential value of therapeutic cloning for type 1 diabetes mellitus cell therapy.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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