Establishment of maturity-onset diabetes of the young-induced pluripotent stem cells from a Japanese patient

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INTRODUCTION
Maturity-onset diabetes of the young (MODY) is a heterozygous monogenic diabetes; more than 13 disease genes have been identified. However, the pathogenesis of MODY is not fully understood, because the pancreatic β-cells of the patients are inaccessible. Therefore, we attempted to establish MODY patient-derived induced pluripotent stem cells (MODY-iPS) cells to investigate the pathogenic mechanism of MODY by inducing pancreatic β-cells. We established MODYS-iPS cells from a Japanese patient with MODYS (R177X), and confirmed that MODYS-iPS cells possessed the characteristics of pluripotent stem cells. In the course of differentiation from MODYS-iPS cells into pancreatic β-cells, we examined the disease gene, HNF1B messenger ribonucleic acid. We found that the amount of R177X mutant transcripts was much less than that of wild ones, but they increased after adding cycloheximide to the medium. These results suggest that these R177X mutant messenger ribonucleic acids are disrupted by nonsense-mediated messenger ribonucleic acid decay in MODY-iPS cells during the developmental stages of pancreatic β-cells.

MATERIAL AND METHODS
Generation of MODY-iPS Cells
Skin fibroblasts were obtained from a MODY5 patient by a 5-mm punch biopsy at Tokyo Women’s Medical University after written informed consent. We purchased healthy women’s fibroblasts from Lonza (Verviers, Belgium) as a control. Aliquots of 10⁶ cells of these control and MODY5-patient skin fibroblasts were transduced with hSOX2, hOCT3/4, hKLF and hCMYC using SeV (MBL, Nagano, Japan) overnight. The cells

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Figure 1 | Characterization of pluripotency of maturity-onset diabetes of the young 5-induced pluripotent stem (MODY5-iPS) cells. (a) Morphology of MODY5-iPS cells. (b) Alkaline phosphatase staining. (c–n) Immunocytochemistry for pluripotency markers. (c) OCT3/4; (e) NANOG; (g) SSEA4; (i) TRA-1-60; (k) TRA-1-81; (d, f, h, j, l) DAPI; (m) SeV, passage 3 (n) SeV, passage 10; (o) karyotype analysis by the G-band method; (p–r) teratoma derived from MODY1-iPS cells; (p) ectoderm – neural tissue; (q) mesoderm – muscle and cartilage; and (r) endoderm – gut tube-like structure.
were washed and cultured in Dulbecco’s modified Eagle medium supplemented 10% fetal bovine serum for 6 days. Then Sev-infected fibroblasts were seeded on mitomycin-treated mouse embryonic fibroblast feeder cells; the next day, the medium was replaced by hiPS medium (Dulbecco’s modified Eagle medium/F12 supplemented with 20% knockout serum replacement, 2 mmol/L L-glutamine, 0.5× penicillin/streptomycin, 1× non-essential amino acids, 55 μmol/L 2-mercaptoethanol and 7.5 ng/mL FGF2). Then, 3–4 weeks later, primary hiPS colonies appeared, and we transferred each colony onto mitomycin-treated SNL feeder cells. These hiPS colonies were maintained on mitomycin-treated SNL feeder cells and enzymatically passaged using CTK solution at 1:5–1:8 once a week. In the present study, three different control or MODY-iPS cell lines were used, respectively. The experiments were carried out with the approval of the ethical committees in Tokyo Women’s Medical

Figure 2 | Differentiation of pancreatic β-cells from maturity-onset diabetes of the young 5-induced pluripotent stem (MODY5-iPS) cells. (a) Scheme of the differentiation process and cloning of disease genes. Definitive endoderm (DE), primitive gut tube (PGT), posterior foregut (PFG) and endocrine progenitor (EP). (b) Expression of marker genes for each stage by reverse transcription polymerase chain reactions (RT–PCR). Undifferentiation (undiff). OAZ1 is a housekeeping gene used as a loading control. (c) Immunocytochemistry of control or MODYS-iPS cells at PGT. (a′–d′) control iPS cells. (e′–h′) MODYS-iPS cells. (a′, e′) HNF1B: green, (b′, d′, f′, h′) DAPI: blue, (c′, g′) SOX17: red.
University and the National Center for Global Health and Medicine.

Detailed Materials and Methods of the items are described in the Data S1.

RESULTS

Establishment of MODY5-iPS Cells

We established MODY-iPS cells using SeV from a Japanese MODY5 patient who had the R177X disease variant, and clinical features were previously characterized. Although the SeV genome was present in the cytoplasm of the MODY5-iPS colonies at passage 3, we confirmed the complete shedding of the SeV genome by passage 10 by immunocytochemistry (Figure 1m) or reverse transcription polymerase chain reaction (RT–PCR; data not shown), indicating that these cells were transgene-free. We used MODY5-iPS cells more than 10 passages for experiments. The MODY5-iPS cells expressed pluripotency markers, such as OCT3/4, NANOG, SSEA4, TRA-1-60 and TRA-1-81, and had alkaline phosphatase activity (Figures 1b–l and S1b–l). Teratoma derived from MODY5-iPS and control hiPS cells contained three germ layers; ectoderm, mesoderm and endoderm (Figures 1p–ra and S1m–o). We confirmed that the karyotypes of the control and MODY5-iPS cells were all normal (Figures 1oa and S1p).

Detection of Disease Gene mRNAs during Differentiation from MODY-iPS Cells into β-Like Cells

The disease gene of MODY5 is HNF1B. Expression of HNF1B was reported at the primitive gut tube stage during pancreatic β-cell development. To detect HNF1B mRNA, control or MODY5-iPS cells were differentiated into pancreatic β-cells using five-stage protocols, and expression of marker genes for each stage was investigated by RT–PCR (Figure 2a, b). We confirmed that HNF1B mRNA as well as Sox17, Foxa2 and HNF4A mRNA were expressed at the primitive gut tube stage (Figure 2b). Immunocytochemistry showed that both control and MODY5-iPS cells differentiated into the primitive gut tube at the same level, because more than 90% of the cells were positive for HNF1B and Sox17 (Figure 2c).

R177X Mutant mRNA Are Destroyed in Differentiated MODY-iPS Cells and Restored by Treatment with Cycloheximide

The mutation site of our MODY5 patient was exon 2 of HNF1B and caused PTC (Figure 3a), which is regarded as a tag of mRNA degradation by NMD. Therefore, we amplified disease gene mRNA by RT–PCR and sequenced them. We confirmed that two bold signals with almost the same strength (C, wild and T, mutant) were present at the mutation site of the genomic sequence of the MODY5-iPS cell (Figure 3b). In contrast to the genomic sequence, one definite signal (C) derived from wild mRNA existed at the mutation site, whereas the signal (T) of R177X mutant mRNA was very weak (Figure 3c), showing that the R177X mutant mRNA might be decayed by the NMD pathway. To confirm this, differentiated MODY-iPS cells were treated with cycloheximide to inhibit NMD. Cycloheximide treatment clearly enhanced the sequence signal of R177X mutant mRNAs compared with non-treated samples (Figure 3c,d). We also confirmed that the sequence of HNF1b mRNA derived from control iPS cells had only clear wild-type transcript signals (Figure 3e).

Figure 3 | Detection of R177X mutation in genomic deoxyribonucleic acid and messenger ribonucleic acid (mRNA). (a) Genomic structure of HNF1B in the maturity-onset diabetes of the young 5 (MODY5) patient. The original start and stop codons existed in exons 1 and 9, respectively. In the case of the R177X mutation, a nonsense mutation was present at exon 2 (C is replaced by T) leading to a premature termination codon (PTC). (b–e) Sequence data for the HNF1B gene and transcripts of control or MODY5-induced pluripotent stem (iPS) cells at the primitive gut tube stage. (b) Genomic sequence data for the MODY5-iPS cells near the mutation site. (c) Sequence data cloned from transcripts derived from MODY5-iPS cells, (d) cycloheximide (CHX)-treated MODY5-iPS cells and (e) control iPS cells.
DISCUSSION
Recently, Caucasian MODY-iPS cells were established by two groups56. However, MODY-iPS cells from Asian patients have never been reported. In the present research, we reported the establishment of MODY-iPS cells from a Japanese MODY5 patient who had the R177X mutation. Among MODY-iPS cells previously established, only MODY2-iPS cells have been used to examine the function of disease gene encoding glycolytic enzyme glucokinase at β-like cells5. We examined the disease gene mRNA encoding transcription factor HNF1B, and confirmed disruption of R177X mutant mRNA with PTC during the developmental process of pancreatic β-cells from MODY-iPS cells for the first time. Although destruction of several MODY3 and MODY5 (not including R177X) mutant mRNAs with PTC was previously reported using ectopic transcripts artificially made from Epstein–Barr virus-transformed lymphoblastoid cells or tubule cells14,15, our results clearly showed that a mutant transcript with PTC is rapidly destroyed by NMD during the developmental stages of pancreatic β-cells, before the onset of MODY.

Thus, MODY-iPS cell technologies make it possible to obtain actual cells expressing MODY genes by differentiating MODY-iPS cells to pancreatic β-cells. Further study will be required for understanding the pathogenesis.

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DISCLOSURE
The authors declare no conflict of interest.

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SUPPORTING INFORMATION
Additional Supporting Information may be found in the online version of this article:

Figure S1 | Characterization of pluripotency of control cells.
Data S1 | Supporting materials and methods.