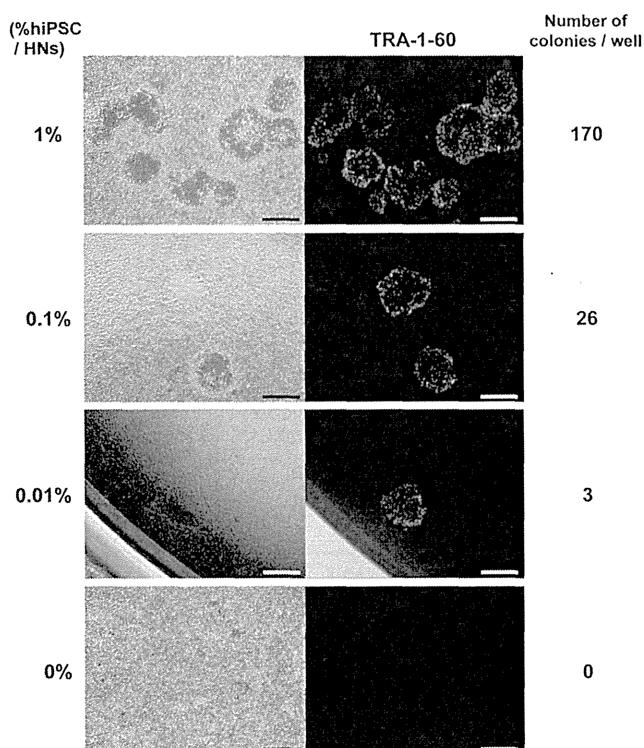

Figure 5. Detection of hiPSCs spiked into human neurons on the culture system using laminin-521 and Essential 8 medium. Morphologies of forming colonies derived from 253G1 cells spiked into human neurons are shown (images in the left). 253G1 cells (1%, 1000 cells; 0.1%, 100 cells; 0.01%, 10 cells; 0%, 0 cells) were spiked into human neurons (100,000 cells) and co-cultured on laminin-521-coated wells in 12-well plates in Essential 8 medium for 6 days. Forming colonies were stained with anti TRA-1-60 antibody (images in the right). HNs, human neurons. Scale bars, 500 μm.
doi:10.1371/journal.pone.0110496.g005

hMSCs, we next tested colony formation of hiPSCs spiked into primary human neurons. Spiked 253G1 cells were co-cultured with human neurons on laminin-521 in Essential 8 medium and clearly formed colonies (Figure 5), which is consistent with the observation using hiPSCs spiked into hMSCs. We detected 170, 26 and 3 colonies that were positive for TRA-1-60 when 253G1 cells were spiked into 1×10^5 human neurons at the ratio of 1, 0.1 and 0.01%, respectively. There was no colony when only human neurons were cultured on our system. These results suggest that this culture system is also useful for detection of trace amounts of hiPSCs not only in hMSCs but also in other types of cells such as human neurons. We also confirmed that no colonies were formed on the well that was not coated with laminin-521 even when human neurons containing 10% hiPSCs were plated (data not shown), indicating that formation of the colonies derived from hiPSCs in human neurons is dependent on laminin-521.

Culture system using laminin-521 and Essential 8 medium has a capacity for direct detection of residual undifferentiated cells contained in differentiating hiPSC cultures

Finally, we examined whether this culture system using laminin-521 and Essential 8 medium is applicable in direct detection of residual hiPSCs contained in differentiated cells derived from hiPSCs. We attempted to differentiate 253G1 cells into MSCs as described in Materials and Methods (Figure 6A). Using this protocol, we observed attached cells with fibroblast-like morphology at the stage of passage 0 MSCs. We confirmed that approximately 20% of these attached cells were positive for staining with anti-CD105 antibody, a MSC marker antibody (Figure S5). During the differentiation process of 253G1 cells into MSCs, we examined the expression levels of residual pluripotency markers in the cell cultures. qRT-PCR analysis revealed that expression of *OCT3/4*, *NANOG* and *LIN28* mRNA were clearly decreased in a time-dependent manner, however, expression levels at the same time point varied markedly among those genes (Figure 6B). In the cells at day 6 of differentiation, mRNA levels of *OCT3/4*, *NANOG* and *LIN28* were 7.3%, 4.8% and 86.4% of the control at day 1, respectively (Figure 6B). At day 14 of differentiation, although *OCT3/4* and *LIN28* were still at detectable levels of 2.6% and 17.2% of control cells, respectively, *NANOG* expression was not detected. These results indicate that the population of residual hiPSCs in differentiating cells, when estimated by the qRT-PCR data, greatly varies and depends on the pluripotency marker gene employed for the estimation. In addition, it is also possible that all the qRT-PCR signals were derived from partially differentiated cells, not from fully undifferentiated cells. To examine colony formation of residual undifferentiated cells in differentiating cell culture, cells at day 6 were dissociated into single cells and replated on laminin-521 in Essential 8 medium. Small cell clusters began to emerge 4 days after plating, rapidly expanded and formed colonies on laminin-521 in Essential 8, while other types of cells gradually decreased their numbers (Figure 6D). After 8 days of culture, 9.5 colonies (the mean of duplicate measurements) were formed from differentiating cells (5×10^4) (Figure 6C) and they were all positive for TRA-1-60 (Figure 6D), indicating that the colonies were derived from residual undifferentiated cells in the differentiating cell cultures. These results suggest that the culture method using a combination of laminin-521 and Essential 8 directly detects residual undifferentiated cells by highly efficient cell amplification. Based on our finding that approximately 0.3 and 6.7 colonies were formed from 1×10^4 MSCs containing 0.01% and 0.1% of 253G1 cells, respectively, in this culture system (Figure S4), and assuming that the sensitivity of the system for hPSCs in EBs are comparable to that in MSCs, the population of the undifferentiated cells in the differentiating cell cultures on day 6 (1.9 colonies/10^4 cells) was estimated to be in between 0.01% and 0.1%. When we tested colony formation using cell cultures on day 14 of differentiation, no colonies were detected on laminin-521 in Essential 8 medium (Figure 6C and data not shown), suggesting that the population of the residual hiPSCs was less than 0.01%.

Discussion

A method to detect residual undifferentiated hPSCs contained in CTPs is required to evaluate product quality during manufacturing processes. In the present study, we propose a novel method to detect a trace amount of undifferentiated hPSCs by highly efficient amplification of those cells *in vitro*. We showed that Essential 8 medium significantly promotes cell growth of hiPSCs dissociated into single cells on laminin-521 compared with the conventional medium, mTeSR1. In addition, Essential 8 medium allowed robust proliferation of hiPSCs even at low cell density on laminin-521. We also demonstrated that 0.001%–0.01% hiPSCs spiked into primary hMSCs were clearly detected and formed colonies on laminin-521 in Essential 8. Similarly, we confirmed that 0.01% hiPSCs spiked into primary human neurons were also detectable on this system. Moreover, we showed that residual undifferentiated hiPSCs contained in differentiating cells were

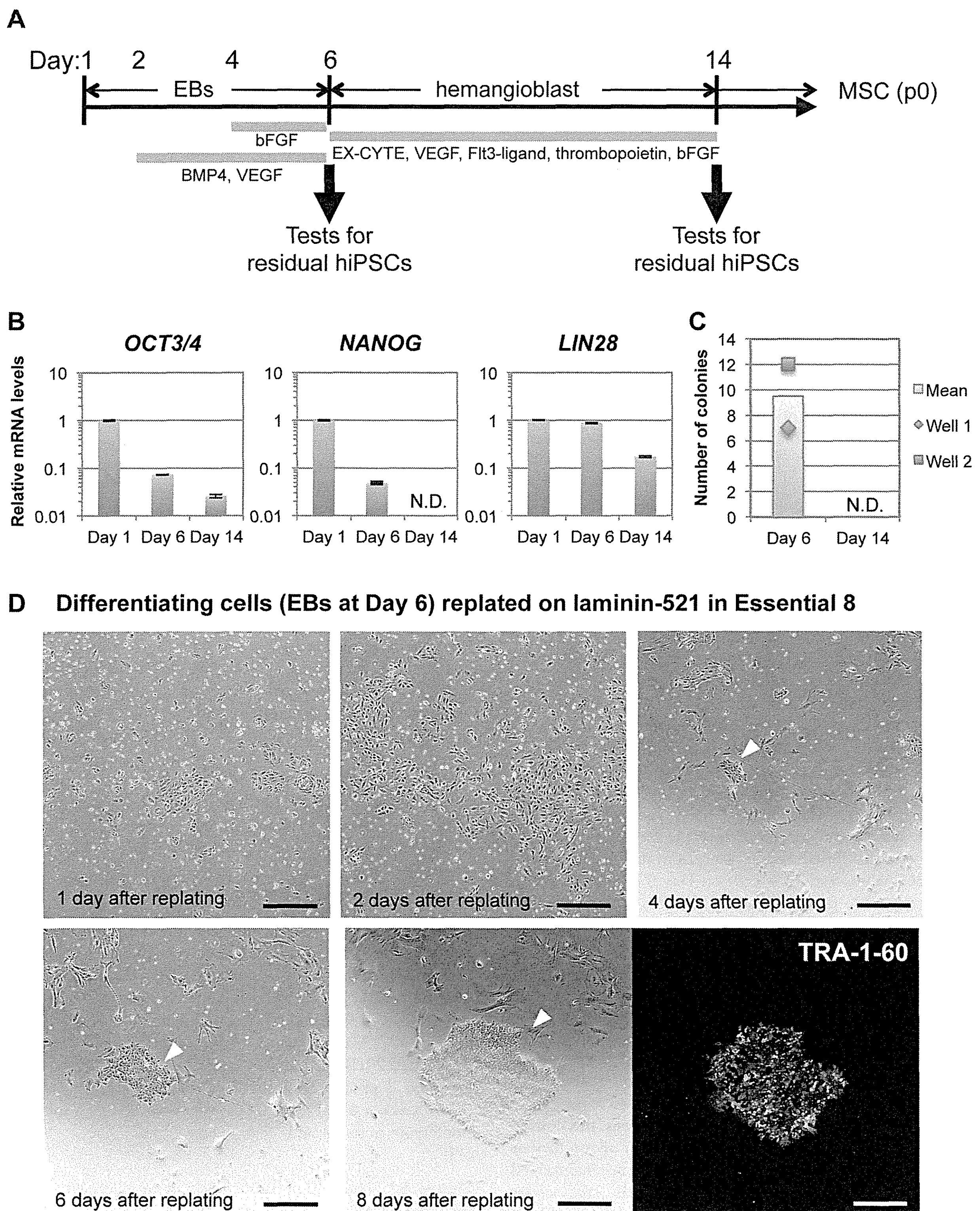

Figure 6. Detection of residual undifferentiated cells contained in differentiating cell cultures. (A) Differentiation scheme of 253G1 cells into MSCs is shown. (B) Expression levels of undifferentiated cell markers (*OCT3/4, NANOG* and *LIN28*) in each cell culture were determined using qRT-PCR. Relative mRNA expression levels are presented as ratios to the level of that in 253G1 cells at Day 1. Results are the mean ± SD (n = 3). (C) Numbers of the forming colonies derived from residual undifferentiated cells in differentiating cell culture at Day 6 or Day 14 are shown. Experiments were carried out in duplicate. Data are present as raw data in each well (shown by plots) or the mean of well 1 and well 2 (shown by bar graphs). (D) Phase contrast images of forming colonies derived from residual undifferentiated cells are shown. Cells at Day 6 of differentiation (EBs) were dissociated into single cells by Accutase and cultured on laminin-521-coated wells in Essential 8 medium (5×10^4/well). After 4 days of culture, small clusters emerged and then started to grow rapidly. Finally, they formed colonies that were positive for TRA-1-60 (shown by immunofluorescence staining, green). Arrowheads indicate a colony derived from same origin. Scale bars, 500 μm.
doi:10.1371/journal.pone.0110496.g006

detectable by forming colonies on laminin-521 in Essential 8 in the process of hMSC differentiation. These results indicate that a culture system utilizing a combination of laminin-521 and Essential 8 medium provides a direct and highly sensitive method for detecting undifferentiated hPSCs. To our knowledge, this is the first report to show a direct and highly-sensitive *in vitro* method for detecting undifferentiated hPSCs as impurities in CTPs.

In this study, highly efficient amplification of undifferentiated hPSCs has been uniquely applied to quality control of CTPs. Amplified hPSC colonies were visible using phase-contrast microscopy and also immunofluorescence staining using pluripotency antibodies, which enabled direct detection of hPSCs contaminating CTPs. Our method distinguished between undifferentiated cells and other cells *in vitro*, and overcame the disadvantage of other *in vitro* methods such as flow cytometry and qRT-PCR. The flow cytometry analysis detects known marker molecules expressed in undifferentiated hPSCs using antibodies and proteins. Signals originating from non-specific detection commonly affect sensitivity of the assay as background. Our *in vitro* method can lower the background arising from non-specific detection and is expected to specifically detect residual undifferentiated hPSCs in CTPs. The qRT-PCR method is highly sensitive and can rapidly quantify undifferentiated cell contamination in CTPs. However, in the present study, gene expression levels of pluripotency markers during the differentiation process of hiPSCs into MSCs varied markedly among those marker genes (Figure 6B). Moreover, there remains a possibility that expression signals of marker genes were not derived from totally undifferentiated hPSCs, but from partially differentiated cells. Indeed, the expression level of *LIN28* did not decrease so much during the differentiation as those of the other genes, which was not obviously associated with the differentiation status of the cells in EBs on Day 6 (Figure 6B), although we have previously reported that *LIN28* was a useful marker for monitoring the level of residual hiPSCs in RPE cells derived from hiPSCs [3]. Thus, it is difficult to determine the presence of residual hiPSCs simply by qRT-PCRs. In contrast, direct detection method using the highly efficient amplification system can clearly detect the presence of intact undifferentiated cells. Based on the result from direct detection of residual hiPSCs when tested the cells on Day 6 of differentiation (approximately 0.01%–0.1%) (Figure 6C-D), it is conceivable that the qRT-PCR signals for the pluripotency marker genes (Figure 6B) are partly derived from residual hiPSCs but mainly derived from partially differentiated cells. Similarly, in the case of the cells at Day 14 of differentiation, the majority of the qRT-PCR signals of *OCT3/4* and *LIN28* (Figure 6B) are considered to be attributable to partially differentiating cells but not to intact hiPSCs. Combination of the *in vitro* methods including our cell culture method would mutually support useful quality assessment of CTPs to detect undifferentiated hPSCs.

In addition to the detection of undifferentiated cells, this culture system using laminin-521 and Essential 8 medium allows further characterization of the undifferentiated cells if they are maintained *in vitro* or inoculated into immunodeficient animals. Analyses for the properties of the residual undifferentiated cells would be necessary not only for the quality assessment of CTPs, but also for improvement of quality specifications of hPSCs as a raw/intermediate material for production of CTPs.

Here, we showed that our culture system is able to detect 0.01% of 409B2 hiPSCs and 0.001% of 253G1 hiPSCs, both of which were spiked into hMSCs (Figure 4). The detection sensitivity for hiPSCs spiked into hMSCs was different between the two hiPSC lines, although such a difference in cell growth on laminin-521 was not found between these two cell lines (Figure 3). This difference may be attributable to the difference in the growth potential of hPSCs in the specific environment provided by CTPs. Kanemura *et al.* have recently demonstrated that hiPSCs co-cultured with iPSC-derived RPE undergo apoptosis by pigment epithelium-derived factor (PEDF) secreted from hiPSC-derived RPE [15], showing that CTPs themselves have the potential to affect cell growth of hPSCs. In the present study, the influence of the co-culture system with hMSCs to the proliferation of hiPSCs might have been different between the two cell lines.

The mechanism by which laminin-521 and Essential 8 medium enhance hiPSCs cell proliferation remains unclear. Rodin *et al.* have recently shown that addition of E-cadherin to laminin-521 permitted the efficient clonal expansion of hESCs [7]. E-cadherin is known to be the primary cell-cell adhesion molecule and essential for hESC survival [16]. We observed that anti-E-cadherin antibody decreased growth potential of hiPSCs under our experimental conditions (data not shown). Therefore, E-cadherin signaling may play some important roles in the rapid cell growth on laminin-521 in Essential 8 medium.

Tumorigenicity is one of the major safety concerns for CTPs derived from hPSCs that are transplanted into patients. However, testing strategies for the tumorigenicity of hPSC-derived CTPs have not yet been established. Here, we introduced a novel testing method for directly detecting a trace amount of undifferentiated hPSCs *in vitro*. The ability of each tumorigenicity-associated test should be taken into consideration to evaluate tumorigenicity of residual undifferentiated hPSCs as impurities in products. *In vivo* tumorigenicity tests using immunodeficient animals can detect tumorigenic cells including undifferentiated hPSCs, but this method is costly and time-consuming. The flow cytometry analysis and qRT-PCR are rapid, but these methods indirectly detect tumorigenic cells depending on marker molecules. Risk of tumorigenicity in hPSCs-derived CTPs should be assessed, based on the results from an appropriate combination of these tumorigenicity-associated tests. Our novel method will contribute to establishment of the testing strategies for tumorigenicity in products, following evaluation of the quality of CTPs derived from hPSCs for the future regenerative medicine/cell therapy.

Supporting Information

Figure S1 (A) Quantification of the number of dissociated 201B7 cells expanded on laminin-521 or Matrigel in Essential 8 or mTeSR1 medium. Data are presented as the mean ± standard deviation (SD) of three independent experiments (***P*<0.01, two-way ANOVA followed by a Bonferroni post-hoc test). LN521, laminin-521. MG, Matrigel. (B) Quantification of the number of dissociated 201B7 cells expanded on laminin-521 or LM511-E8 in Essential 8 or mTeSR1 medium. Results are presented as the mean ±SD (n = 3) (****P*<0.001, two-way ANOVA followed by a Bonferroni post-hoc test).
(TIF)

Figure S2 (A-B) Expression levels of undifferentiated markers (*OCT3/4, NANOG, SOX2* and *LIN28*) in 201B7 cells (A) and 409B2 cells (B) subcultured on laminin-521 in Essential 8 were determined using qRT-PCR. Relative mRNA expression levels are presented as ratios to the level of that in control cells subcultured on Matrigel in mTeSR1 medium by colony passage. Results are presented as the mean ± SD (n = 3). (C-D) Expression levels of markers for the differentiation of embryoid bodies (EBs) derived from 201B7 cells (C) and 409B2 cells (D): endoderm (*GATA6, SOX17*), mesoderm (*CDH5, FOXF1*), and ectoderm (*SOX1, PAX6*). Relative mRNA expression levels are presented as

ratios to the level of that in control cells (EBs at day 10). Results are presented as the mean ± SD (n = 3).
(TIF)

Figure S3 Quantification of the number of 253G1 cells expanded on laminin-521 in Essential 8 or mTeSR1 medium. Cell numbers were counted at day 6, 9, and 12 after plating at 8.0×10^3 cells/cm^2 or 8.0×10^2 cells/cm^2.
(TIF)

Figure S4 Morphologies of forming colonies derived from 253G1 cells spiked into hMSCs are shown (images in the left). 253G1 cells (1%, 300 cells; 0.1%, 30 cells; 0.01%, 3 cells; 0%, 0 cells) were spiked into hMSCs (30,000 cells) and co-cultured on 12-well plates coated with laminin-521 in Essential 8 medium for 9 days. Expression of the undifferentiated cell marker, TRA-1-60, in these colonies was assessed using immunofluorescence staining (images to the right). Each experiment was carried out in duplicate.
(TIF)

Figure S5 Phase contrast images of the cells at day 18 of differentiation (at the stage of passage 0 MSCs) are shown. Expression of MSC marker, CD105, in these cells was examined using immunofluorescence staining (images to the right). Arrowheads indicate the cells that were positive for CD105.
(TIF)

Table S1 Sequences of the primers and probes for qRT-PCR.
(DOCX)

Acknowledgments

This work was supported by Research Grants from the Japanese Ministry of Health, Labour and Welfare (H23-SAISEI-IPPAN-004, H23-SAISEI-IPPAN-005, H24-IYAKU-SHITEI-027, H25-JITSUYOKA(SAISEI)-IP-PAN-008, and Marketing Authorization Facilitation Program for Innovative Therapeutic Products).

Author Contributions

Conceived and designed the experiments: KT SY YS. Performed the experiments: KT. Analyzed the data: KT SY TK HS AU YS. Contributed reagents/materials/analysis tools: KT SY TK HS AU YS. Wrote the paper: KT SY YS. Acquired the funding: HS AU YS.

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Chapter 14

In Vitro Detection of Residual Undifferentiated Cells in Retinal Pigment Epithelial Cells Derived from Human Induced Pluripotent Stem Cells

Takuya Kuroda, Satoshi Yasuda, and Yoji Sato

Abstract

Human pluripotent stem cells (hPSCs) such as human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) are a leading candidate for regenerative medicine/cell therapies because of their capacity for pluripotency and unlimited self-renewal. However, there are significant obstacles preventing the clinical use of hPSCs. A significant safety issues is the presence of residual undifferentiated cells that have the potential to form tumors in vivo. Here, we describe the highly sensitive qRT-PCR methods for detection of residual undifferentiated cells in retinal pigment epithelial (RPE) cells derived from hiPSCs. qRT-PCR using probes and primers targeting *LIN28A (LIN28)* transcripts can detect residual undifferentiated cell levels as low as 0.002 % in hiPSC-derived RPE cells. We expect this method to contribute to process validation and quality control of hiPSC-derived cell therapy product.

Key words Human induced pluripotent stem cells, Retinal pigment epithelial cells, Tumorigenicity, *LIN28*, qRT-PCR

1 Introduction

Pluripotent stem cells such as human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) are pluripotent and have the ability to self-renew. Pluripotency is the ability to differentiate into a variety of cells and self-renewal is the ability to undergo numerous cell division cycles while maintaining cellular identity. Because of these two characteristics, it has been expected that these cell types will provide new sources for robust and continuous production of a variety of cells and tissues for regenerative medicine/cell therapy. Additionally, hiPSCs provide a possible solution to the ethical problems and the immune rejection of hESC-derived cells, thus raising novel avenues for patient-specific cell therapy. As previously reported [1, 2], many attempts are currently under way to differentiate hESCs and hiPSCs into various tissues. Cell therapy using hESCs or iPSCs has already entered

Chrissa Kioussi (ed.), *Stem Cells and Tissue Repair: Methods and Protocols*, Methods in Molecular Biology, vol. 1210,
DOI 10.1007/978-1-4939-1435-7_14, © Springer Science+Business Media New York 2014

the scope of clinical application. Indeed, a clinical trial using hESC-derived-RPE cells for Stargardt's disease and the dry type of age-related macular degeneration (dry AMD) have been initiated [3]. Clinical research using autologous hiPSC-derived RPE for the wet type of age-related macular degeneration (wet AMD) is also being planned in Japan.

One of the most important issues in the development of a safe pharmaceutical or medical device derived from hPSCs is ensuring that the final product does not form tumors after implantation [4]. There are two primary concerns. First, the pluripotent stem cell-based product may be unstable and transform to produce a tumor under some specific environment though the genomic stability of differentiated cells derived from hESCs/hiPSCs is currently unclear. Second, the product derived from human pluripotent stem cells might contain residual undifferentiated stem cells that would eventually proliferate and form a teratoma [5]. To address this second concern, it is critical to develop highly sensitive assays for detection of residual undifferentiated stem cells in the final products, and to determine their lower limit of detection (LLOD). An evaluation study of the in vivo tumorigenicity assay using severe combined immunodeficiency (SCID) mice has shown that 245 undifferentiated hESCs spiked into 10^6 feeder fibroblasts produce a teratoma [6]. However, some in vitro assays, such as quantitative real-time polymerase chain reaction (qRT-PCR), flow cytometry, and immunohistochemistry, have been used to indicate the undifferentiated state of stem cells with various markers (such as *POU5F1 (OCT3/4)*, *NANOG*, *SOX2*, *LIN28*, *MYC* (*C-MYC)*, *REX1*, *KLF4*, *TRA-1-60*, *TRA-1-81*, *SSEA-3*, and *SSEA-4*) [7, 8]. We have demonstrated that the qRT-PCR assay can successfully detect as low as 0.002 % residual undifferentiated hiPSCs in hiPSC-induced RPE cells using *LIN28* as a target gene (designated as the *LIN28/* qRT-PCR method) [9]. To the best of our knowledge, the *LIN28/* qRT-PCR method is the most sensitive of the previously reported other methods in detecting undifferentiated PSCs. In this chapter, the *LIN28*/qRT-PCR protocol used in our laboratories is described in detail.

2 Materials

1. Pipetman (2, 10, 20, 200, and 1,000 μL).
2. Incubator, humidified, 37 °C, 5 % CO_2.
3. Sterile biosafety cabinet.
4. Liquid disposal system for aspiration.
5. Centrifuge.
6. Sampling tubes (1.5 mL).

7. Sterile serological pipets (5, 10, and 25 mL).
8. Sterile conical tubes (15 and 50 mL).
9. Cell culture plates (60 and 100 mm).
10. Tips (10, 20, 200, and 1,000 μL).

2.1 Maintenance and Culture of hiPS, RPE Cell, and SNL Cell

1. The hiPSC line 201B7 induced by transducing *OCT3/4*, *SOX2*, *KLF4*, and *C-MYC* (RIKEN Cell Bank).
2. Normal human retinal pigment epithelial cells (Lonza Biologics, 00194987).
3. SNL cells (a mouse fibroblast STO cell line expressing the neomycin-resistance gene cassette and LIF, CELL BIOLABS, CBA-316).

2.2 Cell Culture

2.2.1 SNL Culture

1. Dulbecco's modified Eagle medium (DMEM).
2. Phosphate-buffered saline (PBS), calcium free, magnesium free.
3. Fetal bovine serum (FBS).
4. L-Glutamine.
5. Penicillin/streptomycin.
6. Gelatin: To make 0.1 % gelatin solution, dissolve 1.0 g of gelatin powder in 1,000 mL of distilled water, autoclave, and store at 4 °C.
7. SNL medium, 500 mL: Mix 500 mL DMEM, 38.5 mL FBS, 5.5 mL L-glutamine, and 3 mL penicillin/streptomycin.
8. 0.25 % Trypsin/EDTA solution.
9. Mitomycin C.

2.2.2 hiPSC Culture

1. Primate ES Cell medium (ReproCELL, RCHEMD001).
2. Penicillin/streptomycin.
3. Recombinant human FGF basic 146 aa (bFGF).
4. hiPSC medium, 500 mL: Mix 500 mL primate ES cell medium and 2.5 mL penicillin/streptomycin. Add 4 ng/mL bFGF into the medium before use. Store at 4 °C for up to 2 weeks.
5. PBS (*see* Subheading. 2.2.1).
6. CTK solution (ReproCELL, RCHETP002).
7. StemPro EZPassage.
8. Cell scraper.
9. 100 μm cell strainer.

2.2.3 Primary PRE Cell Culture

1. RtEBM Bullet Kit (RtEBM medium; Lonza).
2. PBS (*see* Subheading 2.2.1).
3. 0.25 % Trypsin/EDTA solution.
4. FBS (contained in RtEBM Bullet Kit).

2.3 Total RNA Extraction

1. RNeasy Mini Kit.
2. QIAshredder.
3. RNase-free DNase set.
4. Cell scraper.

2.4 qRT-PCR

1. QuantiTect Probe one-step RT-PCR Kit.
2. 96-Well PCR plate.
3. Dual-labeled probe and primer.
4. TaqMan GAPDH control reagents, human.
5. Applied Biosystems 7300 Real Time PCR systems.

3 Methods

3.1 Cell Culture

3.1.1 MMC-Treated SNL Cells

1. Add 3 or 5 mL of gelatin solution into a 60 or a 100 mm dish and incubate for 30 min.
2. Aspirate gelatin solution and use this dish as gelatin-coated dish.
3. Thaw quickly the 1×10^6 cells of cryopreserved SNL cells in water bath at 37 °C and suspend with 9 mL of SNL medium in a 15 mL tube.
4. Centrifuge the 15 mL tube a $170 \times g$ for 5 min and aspirate supernatant.
5. Suspend SNL cell pellet with 10 mL of SNL medium and transfer into a gelatin-coated 10 cm dish.
6. Culture in a humidified atmosphere of 95 % air with 5 % CO_2 at 37 °C (*see* **Note 1**).
7. When SNL cells reach confluence, add 12 μg/mL MMC and incubate for 2 h and 30 min.
8. Wash the MMC-treated SNL cells twice with 10 mL of PBS.
9. Detach the cells with 1 mL of 0.25 % trypsin/EDTA solution, add 10 mL of SNL medium, and then transfer into 15 mL tube.
10. Centrifuge the 15 mL tube at 170 g for 5 min and aspirate supernatant.
11. Suspend the cell pellet with 10 mL SNL medium, transfer the cell suspension to a 60 mm dish (5×10^5 cells/dish), and incubate in a humidified atmosphere of 95 % air with 5 % CO_2 at 37 °C for 1 day.

3.1.2 hiPSC Culture on Feeder Cells

1. Culture hiPSC colonies in 60 mm dish (pre-seeded with MMC-SNL feeder cells) until colonies reach 70–80 % confluency. Aspirate the medium, and wash twice the cells with 5 mL of PBS per dish.

2. Remove PBS completely, add 0.5 mL CTK solution, and incubate at 37 °C for 3 min (*see* **Note 2**).
3. Aspirate CTK solution, and wash twice with 5 mL of PBS.
4. Remove PBS completely and add 5 mL of hiPSC medium.
5. Cut hiPSC colonies using EZPassage (*see* **Note 3**).
6. Detach hiPSC colonies using a cell scraper.
7. Resuspend the cell clump in 5 mL of hiPSC medium and through 100 μm cell strainer.
8. Transfer the cell clump suspension to a 15 mL tube. Centrifuge at $100 \times g$ for 5 min, and then aspirate the supernatant.
9. Resuspend the cell clump in 5 mL of hiPSC medium and transfer the cell clump suspension onto a 60 mm dish that is pre-seeded with MMC-SNL feeder cells.
10. Incubate at 37 °C and 5 % CO2 until cells reach 70–80% confluency (*see* **Note 4**).

3.1.3 Primary RPE Cell Culture

1. Culture primary RPE cells in 100 mm dish for 14 days.
2. Aspirate the medium, and wash the cells with 10 mL PBS.
3. Remove PBS completely, add 0.5 mL 0.25 % trypsin/EDTA solution, and incubate at 37 °C for 10 min. Add 10 mL RtEBM medium (with 2 % FBS), and then transfer the cell suspension to a 15 mL tube.
4. Centrifuge at $200 \times g$ for 5 min, and then discard the supernatant.
5. Resuspend the cell in 5 mL of RtEBM medium (with 2 % FBS).
6. Seed 1×10^6 cells into a 60 mm dish (*see* **Note 5**).

3.2 RPE Cell Differentiation of hiPSCs

The procedure for differentiating hiPSCs into RPE cells was performed as previously described [9, 10].

3.3 Total RNA Extraction

1. Extract total RNA using the RNeasy Mini Kit, according to the manufacturer's instructions (*see* **Note 6**).
2. The lysate was homogenized using a QIAshredder and on-column DNase digestion was performed according to the manufacturer's instructions.
3. The RNA quantity and quality were determined by measuring the absorbance at 260 and 280 nm using NanoDrop. The RNA concentration was calculated and the RNA samples were stored at −80 °C.

3.4 One-Step qRT-PCR

1. PCR primers and dual-labeled probes are listed in Table 1.
2. Preparation of a reaction mix using the QuantiTect Probe RT-PCR kit. The primer and probe concentrations were

Table 1
qRT-PCR probes and primers [9]

Gene	Probe sequences (5′ → 3′)	Forward primer sequences (5′ → 3′)	Reverse primer sequences (5′ → 3′)
NANOG	TGCTGAGGCCTTCTGCGTCACACC	CTCAGCTACAAACAGGTGAAGAC	TCCCTGGTGGTAGGAAGAGTAAA
OCT3/4	CGGACCACATCCTTCTCGAGCCCAAGC	GAAACCCACACTGCAGCAGA	TCGCTTGCCCTTCTGGCG
LIN28	CGCATGGGGTTCGGCTTCCTGTCC	CACGGTGCGGGCATCTG	CCTTCCATGTGCAGCTTACTC
REX1	AGCAAACACCTGCTGGACTGTGAGCAC	CCATCGCTGAGCTGAAACAAA	CCTCCAGGCAGTAGTGATCTG
C-MYC	TTGTTCCTCCTCAGAGTCGCTGCTGGT	GCTCCATGAGGAGACACCG	CCACAGAAACAACATCGATTTCTTC
SOX2	CTCGCAGACCTACATGAACGGCTCGC	GCGCCCTGCAGTACAACTC	CGGACTTGACCACCGAACC
KLF4	ACCTGCGAACCCACACAGGTGAGAAC	CCTACACAAAGAGTTCCCATCTCA	CCGTCCCAGTCACAGTGGTA

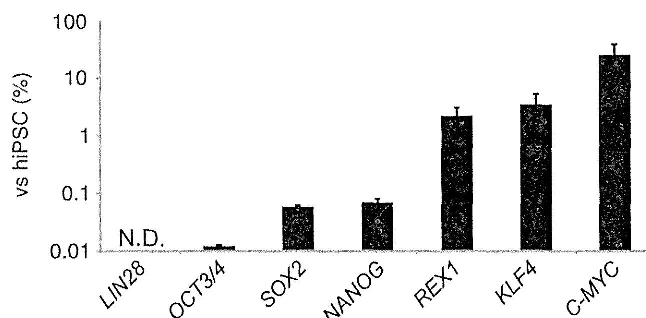

Fig. 1 The relative mRNA expressions in primary RPE cells of *LIN28*, *OCT3/4*, *SOX2*, *NANOG*, *REX1*, *KLF4*, and *C-MYC* were determined by qRT-PCR analysis [9]. All values are expressed as mRNA levels relative to those in undifferentiated hiPSCs. Results are means ± standard deviation ($n = 3$)

determined to be 400 nM and 100 nM, respectively, in a final reaction volume of 25 μl. 250 ng of total RNA (5 μL of 50 ng/μL total RNA) was used as template (*see* **Note** 7). Prior to amplification, the contents of each reaction were mixed by spinning the plate for 2 min at 700 × *g*. The optimal thermal cycling conditions were 50 °C for 30 min and 95 °C for 15 min followed by 45 cycles of 94 °C for 15 s and 60 °C for 1 min.

3.5 Quantification

Data analysis based on a standard curve method: A 201B7 total RNA dilution series (10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003, and 0.001 ng/μL) was prepared as a standard curve.

Target gene expression levels were normalized to those of the GAPDH transcript, which were quantified using TaqMan human GAPDH control reagents.

3.6 Detection of Undifferentiated hiPSCs Using qRT-PCR

3.6.1 Identification of Highly Selective Markers for Undifferentiated hiPSCs

To identify highly selective markers for undifferentiated hiPSCs, we compared the *OCT3/4*, *KLF4*, *C-MYC*, *SOX2*, *NANOG*, *LIN28*, and *REX1* mRNA levels in hiPSCs and primary RPE cells (Fig. 1). Primary RPE cells were found to endogenously express *C-MYC* at 25.49 % of the levels observed in hiPSC, which was consistent with the previous finding that MEF expressed C-*MYC* at approximately 20 % of levels observed in mouse ES cells [11]. *KLF4* and *REX1* expression levels in primary RPE cells were 3.51 % and 2.23 %, respectively, compared with hiPSCs. However, there was more than a 1,000-fold difference in *NANOG* (0.07 %), *SOX2* (0.06 %), *OCT3/4* (0.01 %), and *LIN28* (not detected) gene expression between primary RPE cells and hiPSCs. These results suggested that the latter four genes are useful in detecting hiPSCs in RPE cells.

3.6.2 hiPSC-Spiked Sample Analyzed Using the Lin28/qRT-PCR Method

We then conducted two sets of qRT-PCR assays to evaluate the utility of these marker genes to detect spiked hiPSCs in primary RPE cells. We first measured *NANOG*, *OCT3/4*, and *LIN28* mRNA levels in five lots of primary RPE cells (the negative control)

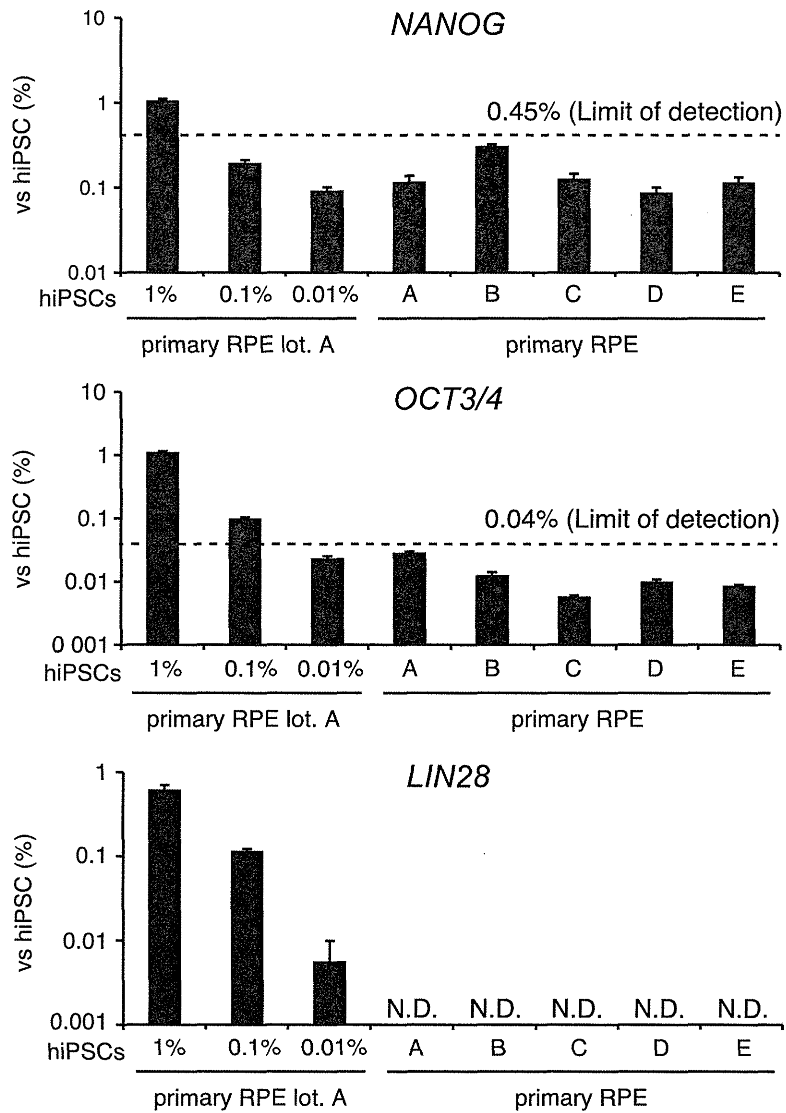

Fig. 2 qRT-PCR analysis of hiPSCs spiked into primary RPE cells and five lots of primary RPE cells [9]. Single-cell hiPSCs were spiked into primary RPE cells, and total RNA was isolated from the mixed cells. The mRNA levels of *NANOG*, *OCT3/4*, and *LIN28* are shown as a relative expression. Limit of detection was calculated as the mean plus 3.3-fold the standard deviation of the measurement of the five lots of primary RPE cells. All values are expressed as mRNA levels relative to those in undifferentiated hiPSCs. Results are means ± standard deviation ($n = 3$)

to determine the LLOD. Limit of detection was calculated as the mean plus 3.3-fold the standard deviation of the measurement of the five lots of primary RPE cells [12]. The LLOD for *NANOG* and *OCT3/4* mRNA was 0.45 % and 0.04 %, respectively, compared with hiPSCs (Fig. 2). The LLOD for *LIN28* could not be calculated because no fluorescent signal for *LIN28* expression was detectable in the primary RPE cells. These results along with

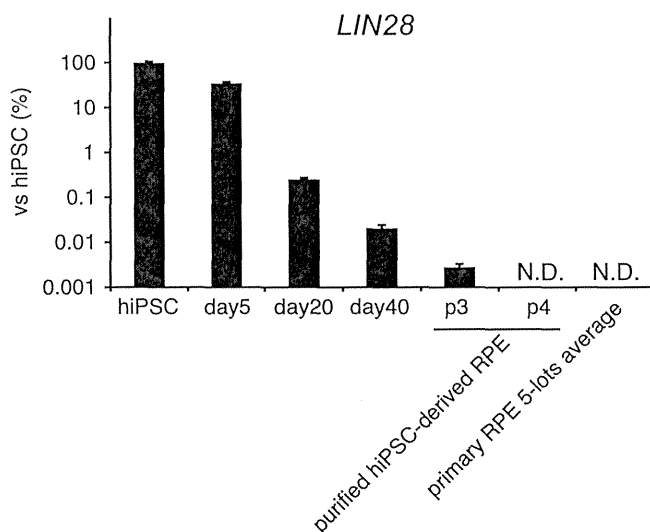

Fig. 3 *LIN28* expression of hiPSCs differentiating into RPE and purified hiPSC-derived RPE cells (passages 3 and 4) [9]. All values are expressed as mRNA levels relative to those in undifferentiated hiPSCs. Results are means ± standard deviation ($n = 3$)

experiments spiking 2.5×10^4 (1 %), 2.5×10^3 (0.1 %), and 2.5×10^2 (0.01 %) hiPSCs into 2.5×10^6 primary RPE cells (Fig. 2) indicated that measuring *NANOG*, *OCT3/4*, and *LIN28* expression using qRT-PCRs detected at least 1 %, 0.1 %, and 0.01 % contamination of residual undifferentiated hiPSCs in RPE cells, respectively.

3.6.3 hiPSC-Derived RPE Analyzed Using the LIN28/ qRT-PCR Method

We examined whether qRT-PCR analysis of *LIN28* was able to detect residual undifferentiated cells in differentiated RPE cells from hiPSCs. Total RNA extracted from the differentiating cells (days 5, 20, and 40) and purified hiPSC-derived RPE cells (passages 3 and 4) underwent qRT-PCR analysis. The *LIN28* mRNA level was continuously downregulated during the differentiation process, being 0.02 % that of hiPSCs at day 40. In early passage culture (passage 3) after purification, *LIN28* was still significantly expressed at a level 0.002 % that of hiPSCs. From passage 4 onward, however, there was no detectable level of *LIN28* expression observed in hiPSC-derived RPE cells (Fig. 3). These results suggest that the *LIN28*/qRT-PCR method detects 0.002 % of residual undifferentiated hiPSCs in hiPSC-induced RPE cells, which indicates that a single hiPSC in 5.0×10^4 RPE cells is detectable.

4 Notes

1. Change the medium every 3 days until the next passage step.
2. To prevent inactivation of CTK, do not warm 37 °C before use. Make sure that feeder cells are dissociated, while hiPS colonies

remain attached on the plate. Then, remove CTK solution as soon as possible to maintain good viability of the harvested cell.

3. Cuts hiPS colonies into uniform-sized pieces for reproducible and optimal passaging.
4. Change the medium every day until the next passage step.
5. Next day, make sure that RPE cells are attached on plate, and change the medium with RtEBM medium (without FBS). Change the medium every 2–3 days until the next passage step.
6. In the spiking study, 201B7 and RPE cells were mixed at a defined cell number, before total RNA extraction.
7. If sufficient total RNA sample were not available, it is possible to use 50 ng total RNA as qRT-PCR template.

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2014年度
日本再生医療学会

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ヒト多能性幹細胞加工製品に残存する未分化多能性幹細胞の高感度検出法の開発

Development of highly sensitive methods for detection of residual undifferentiated pluripotent stem cells in products derived from processing of human pluripotent stem cells

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Summary

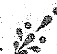
Human pluripotent stem cells (hPSCs), e.g. human embryonic stem cells and human induced pluripotent stem cells (hiPSCs), have promising potential as raw materials of cell-based therapeutic products. However, there are some technical challenges that must be overcome before their clinical use. One of the challenges is the lack of highly sensitive methods for detection of residual undifferentiated hPSCs that have tumorigenic potential. We characterized and established *in vitro* assays for detection of tumorigenic/undifferentiated cells in hiPSC-derived products. The soft agar colony formation assay appeared unable to detect hiPSCs, presumably attributable to dissociation-induced apoptosis, a unique property of hPSCs. The flow cytometry assay using anti-TRA-1-60 antibody detected 0.1% undifferentiated hiPSCs that were spiked in primary retinal pigment epithelial (RPE) cells. Moreover, qRT-PCR was found to detect a trace amount of *LIN28* mRNA, which is equivalent to that present in a mixture of a single hiPSC and about 50,000 RPE cells. Our studies provide highly sensitive and quantitative *in vitro* assays for safety/quality profiling of hPSC-derived products.

KEY WORDS
iPS細胞 再生医療 造腫瘍性 安全性 品質管理

はじめに

現在，ヒトES細胞やヒトiPS細胞といった多能性幹細胞に由来する移植細胞を用いた再生医療・細胞治療の開発が世界中で広く展開されており，難病治療への期待が高まっている。しかし，ヒト多能性幹細胞由来移植細胞に関する汎用性の高い品質・安全性評価方法の開発とその体系化・標準化が大きく立ち遅れており，実用化における隘路となっている。特に大きな問題としては，移植細胞中に残存する未分化なヒト多能性幹細胞に起因する製品の造腫瘍性の評価法・管理法が確立されていないことが挙げられる。こうした方法の確立なしには，どのようなヒト多能性幹細胞由来移植細胞であれ，実用化・産業化を達成することは非常に難しい。

本稿では，ヒト多能性幹細胞由来移植細胞の品質・安全性の確保の上で重要な，未分化細胞の残存の評価方法に関し，ヒトiPS細胞由来網膜色素上皮細胞での例を中心に我々のこれまでの経験を紹介する。[本稿は，第13回日本再生医療学会総会(平成26年3月)でのJohnson & Johnson Innovation Award受賞講演をもとに記述したものである。]



多能性幹細胞由来移植細胞の造腫瘍性

「造腫瘍性」(tumorigenicity)とは，動物に移植された細胞集団が増殖することにより腫瘍を形成する能力をいう[1]。ヒトES細胞やヒトiPS細胞を樹立した時には，細胞の多能性(多分化能)を確認する目的で，免疫不全動物に細胞を移植し，その体内でのテラトーマ(teratoma；奇形腫)の形成，すなわち移植した細胞が3つの胚葉系の様々な細胞種に分化することを確認する。つまり，ヒト多能性幹細胞は造腫瘍性を元来の特性として持っていることになる。この点がヒト体細胞・体性幹細胞と大きく異なる。したがって，ヒト多能性幹細胞由来移植細胞においては，未分化な多能性幹細胞の混入・残留により異所性組織形成や腫瘍形成が惹起されるリスクがある。このことは，移植細胞中に残存する未分化な多能性幹細胞を可能な限り除去する工夫が必要であることを意味し，実際，その取り組みに関する報告はこれまでに多く存在する[2)-7)]。しかし，忘れられがちだが，ヒト多能性幹細胞由来移植細胞の実用化・品質評価においては，実際に未分化な多能性幹細胞が除去できたかを確認する手段，すなわち未分化な多能性幹細胞の混入・残留量を高感度で確認する方法も重要であり，必要である。こうした方法の開発は，多能性幹細胞から特定の細胞種への効率的な分化誘導法の開発や，残存未分化多能性幹細胞の除去方法の開発などに比べて著しく立ち遅れており，ヒト多能性幹細胞由来移植細胞を用いた再生医療・細胞治療の実現における隘路として残されたままの状態にある。

多能性幹細胞・造腫瘍性細胞の検出

ヒト多能性幹細胞由来移植細胞は，通常，目的とする細胞以外にその前駆細胞や残存多能性幹細胞およびその他の目的外細胞が含まれていると考えられる。こうした細胞集団における造腫瘍性に関するリスクファクターとしては，「ヒト多能性幹細胞の残存」および「造腫瘍性形質転換細胞の出現・混入」の2点が挙げられる。

「ヒト多能性幹細胞の残存」に関しては，多能性幹細胞のマーカー遺伝子ないしマーカー蛋白質を利用した方法により評価することが可能である。手技としては後述する定量RT-PCR(qRT-PCR)やフローサイトメトリーなどが挙げられる。一方,「造腫瘍性形質転換細胞の出現・混入」を評価するための方法としては，例えば細胞増殖特性の評価(不死化細胞の検出)や軟寒天コロニー形成試験(足場非依存性増殖細胞の検出，後述)が挙げられる。造腫瘍性細胞の検出を目的とした方法として，*in vivo*造腫瘍性試験系(製品を免疫不全動物に移植して腫瘍形成を評価する方法)を活用することも考えられるが，製品の中に含まれるわずかな造腫瘍性細胞を検出する必要があるため，十分に高い感度を備えている必要がある。検出感度の高い試験系としては，NOD/SCID/γCnull(NOG)など，ヌードマウスよりも免疫力の低下した重度免疫不全マウス系統を利用することが考えられる[8)-10)]。ただし新規免疫不全動物モデルを用いた方法は，高価かつ時間がかかる点が問題とされ，また評価方法が未整備であり，その標準化・体系化が課題となっている。

造腫瘍性関連*in vitro*試験[11)]

細胞集団の造腫瘍性，あるいは細胞集団中の造腫瘍性細胞を検出する系としていくつかの*in vitro*試験系がある。それぞれの長所と短所を*in vivo*試験法と併せて表にまとめた。なお，核型分析などの染色体異常を検出することを目的とする試験については，技術的に確立されているものの，得られた結果と細胞の造腫瘍性との相関性が低いことが多く，最終製品の非臨床安全性という面での造腫瘍性の評価よりも，原材料の品質の安定性，または製造過程における製品の遺伝的安定性を評価する目的で実施されるべきものと言える。

軟寒天コロニー形成試験は，がん細胞の多くが足場非依存的に増殖するのに対して，接着性正常細胞は足場が存在しないとアポトーシス(アノイキス)を起こすという性質を生かした試験系で，細胞を分散して軟寒天に封入し，足場非依存的な細胞増殖を検出する試験である。この試験系では，細胞のクランプ残存や寒天

表 造腫瘍性関連試験の比較

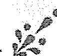
試験法	軟寒天コロニー形成試験	フローサイトメトリー	qRT-PCR	*in vivo* 造腫瘍性試験※
目的	足場非依存的増殖（悪性形質転換細胞）の検出	未分化な多能性幹細胞の検出	未分化の多能性幹細胞の検出	悪性形質転換細胞および未分化な多能性幹細胞の検出
期間	30日	1日	6時間	12～16週間
利点	●安価	●短時間・簡便 ●個々の細胞を解析	●迅速 ●簡便 ●定量的 ●高感度	●直接的 ●微小環境での造腫瘍性を評価できる
欠点	●間接的 ●浮遊系細胞には使えない ●ヒトiPS細胞検出には使用不可能（分散誘導性細胞死）	●間接的 ●既知のマーカー分子を発現する細胞以外は検出不能 ●ゲーティングが結果に影響	●間接的 ●既知のマーカー分子を発現する細胞以外は検出不能	●費用と時間がかかる
下方検出限界	網膜色素上皮細胞中の1%のPA-1細胞（ヒトテラトカルシノーマ由来細胞）	網膜色素上皮細胞中の0.1%のiPS細胞マーカー：TRA-1-60	網膜色素上皮細胞中の0.002%以下のiPS細胞マーカー：*LIN28*	10^6個のフィーダー細胞中に含まれる245個（0.02%）の未分化ES細胞

（※Hentze H, et al : Stem Cell Res 2 : 198-210, 2009）

中での凝集による足場依存的細胞増殖を防ぐという意味で，単一細胞への分散が重要である。一方，ヒトES細胞やヒトiPS細胞はトリプシン処理などによる分散によってアポトーシスを起こす特異な性質を持つことが知られている。そこで，単一細胞に分散したヒトiPS細胞の軟寒天培地中での増殖を検討したところ，分散誘導性アポトーシスを抑制するといわれているROCK阻害剤Y-27632存在下であっても，軟寒天培地中での増殖は認められなかった。すなわち，軟寒天コロニー形成試験は未分化なヒト多能性幹細胞の混入を検出する目的には不適であるということが確認された。次に，モデルケースとして初代培養ヒト網膜色素上皮細胞（RPE細胞）にヒト卵巣テラトカルシノーマ細胞株PA-1を一定量添加した標本を用い，正常細胞中への悪性形質転換細胞の混入に関する軟寒天コロニー形成試験の検出限界を検討した。その結果，混入するPA-1細胞を検出するには，混入率がRPE細胞の数の1％以上必要であることが明らかとなった。

特定のマーカー蛋白質を指標に未分化ヒトiPS細胞を検出する系として，フローサイトメトリーがある。正常細胞のモデルケースとして初代培養RPE細胞を用いて我々が検討したところでは，未分化細胞マーカーとされるOct3/4，Sox2およびTRA-1-60に対する特異的抗体によって，未分化iPS細胞と分化細胞とが峻別できることが確認された。初代培養RPE細胞にiPS細胞をスパイクする実験により，正常細胞（初代培養RPE細胞）に混入する未分化細胞の検出限界を検討した結果，0.1％以上の混入量であれば有意な検出シグナルが得られることが明らかとなった。

qRT-PCRは，特定のマーカー遺伝子発現を指標に未分化ヒトiPS細胞を検出する高感度な系であるが，どのマーカーが最適であるか，そして検出感度はどのくらいか，ということに関する情報はこれまでほとんどなかった。そこで我々は，未分化細胞に選択的に発現するとされる遺伝子として*OCT3/4*，*KLF4*，*C-MYC*，*SOX2*，*NANOG*，*LIN28*，*REX1*を選び，これらの中でどの遺伝子発現が未分化細胞の混入指標として最適か，最適な遺伝子を使用した場合の混入未分化細胞の検出限界はどのくらいかを検討した。正常細胞のモデルケースとしては，軟寒天コロニー形成試験およびフローサイトメトリーの時と同様，初代培養RPE細胞を用いた。検討の結果，初代培養RPE細胞



であっても，*C-MYC*, *KLF4*および*REX1*は，ヒトiPS細胞での発現を100％とした場合，数％～25％のレベルで発現していることが明らかとなった。しかしながら，患者1回あたりの投与量が10^4～10^9とされるiPS細胞由来移植細胞の中にわずかに残留するiPS細胞の検出を行うには，この程度の選択性では全く不十分である。一方，*OCT3/4*, *SOX2*, *NANOG*, *LIN28*の初代培養RPE細胞における遺伝子発現量は，対iPS細胞比で1/1,000未満であった。RPE細胞にiPS細胞をスパイクして検討した結果，*OCT3/4*, *SOX2*, *NANOG*を指標にしたiPS細胞の検出限界はそれぞれ，0.01％，0.06％，0.07％であった。*LIN28*については，初代培養RPE細胞中の発現が全く検出されなかったため，「ネガティブコントロールのシグナル値の平均値＋標準偏差の3倍」という通常の方法により下方検出限界を求めることはできなかった。しかしながら，スパイク実験およびヒトiPS細胞由来RPE細胞を用いた検討から，0.002％程度のiPS細胞が混入した際にも*LIN28*の有意な発現シグナルが観測されることが明らかとなっている。すなわち，*LIN28*を指標にすれば，約50,000個RPE細胞中に1個の割合で混入する未分化iPS細胞を検出できることになる。この方法は，我々の知り得る限り，分化細胞中の残存ヒトiPS細胞の検出方法としては，学術論文として公表されている方法の中で最も感度が高く，平成25年8月から開始されている神戸の理化学研究所と先端医療センター病院の自己iPS細胞由来RPE細胞を用いた臨床研究計画の中でも品質管理試験として採用されるに至っている。

おわりに

再生医療製品を対象にした造腫瘍性試験ガイドラインは未だに存在しない。したがって，現時点では，再生医療製品の中でも特に造腫瘍性に関して懸念の強いヒトiPS細胞由来移植細胞をはじめとする製品については，本稿で挙げたような，タイプの異なる試験を複数実施し，総合的に判断すべきであると考えられる。なお，個別の製品で示すべき具体的評価事項は，原材料や製品の特性・対象疾患・リスクマネジメントプランなどを勘案して製品ごとに判断されるものである。なお，適切な試験(を組み合わせた)結果・評価についても，ヒトでの結果を完全に保証するものではなく，各試験法の能力と限界を理解した上で，リスク評価・リスクマネジメント立案およびインフォームド・コンセントの受領を行うことが重要であると考えられる。

謝　辞

本研究を遂行するにあたり，多大なるご指導をくださいました西川伸一先生，川真田伸先生，松山晃文先生，鄒正博先生をはじめとする先端医療振興財団の先生方および高橋政代先生をはじめとする理化学研究所の先生方，品質・安全性の考え方について多くのご示唆を下さいました近畿大学 早川堯夫先生，一丸となって多くの実験に取り組んでくださった安田智先生，黒田拓也先生，草川森士先生他，国立医薬品食品衛生研究所遺伝子細胞医薬部のメンバーに深く感謝いたします。

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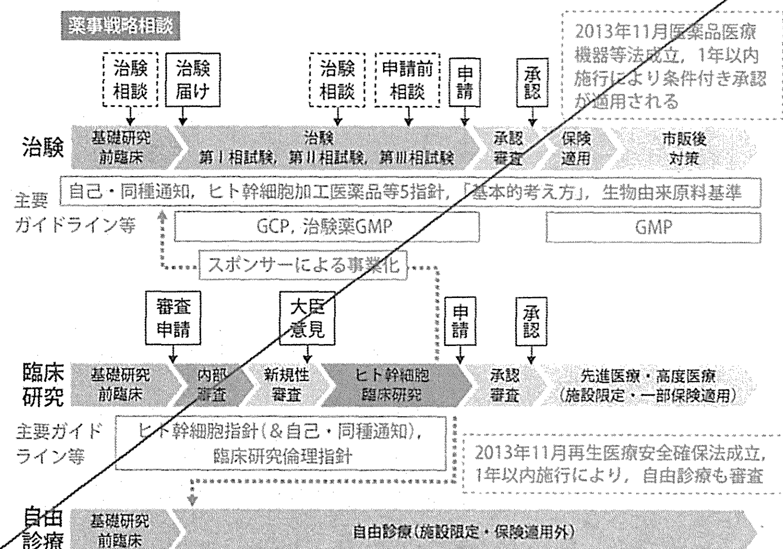
(8))，といった項目が盛り込まれた。これらをさらに（自己／同種）体性幹細胞，（自己／同種）iPS（様）細胞，ES 細胞加工製品に特化した五つの指針（薬食発 0907 第 2 号,薬食発 0907 第 3 号，薬食発 0907 第 4 号，薬食発 0907 第 5 号，薬食発 0907 第 6 号）が，2012 年 9 月 7 日に通知された。

一方，医療行為としての再生医療を裏打ちする 2006 年 7 月 3 日の「ヒト幹細胞を用いる臨床研究に関する指針（全面改訂版 2010 年 11 月 1 日厚生労働省告示第 380 号：2013 年 10 月厚生労働省告示第 371 号)」，2010 年 3 月 30 日の「医療機関における自家細胞・組織を用いた再生・細胞医療の実施について」(医政発 0330 代 2 号)，翌年 4 月 28 日の「再生・細胞医療に関する臨床研究から実用化への切れ目のない移行を可能にする制度的枠組みについて」(医政発 0428 第 11 号；医薬発 0428 第 4 号)，などが発出された。内閣は 2012 年 6 月 6 日に医療イノベーション 5 カ年戦略を策定，厚生労働省・文部科学省・経済産業省が互いに連携し，再生医療の迅速な実現に向け，基礎から臨床にわたるシームレスな研究開発の支援と審査体制の強化（PMDA の増員など)，承認の迅速化，再生医療関連産業の基盤整備と振興といった方針を打ち出した。

さらに 2013 年 4 月 26 日，議員立法の「再生医療を国民が迅速かつ安全に受けられるようにするための施策の統合的な推進に関する法律（再生医療推進法)」が，11 月 20 日には「再生医療等の安全性の確保等に関する法律（再生医療法)」と，再生医療等製品を医薬品や医療機器と区別し，その特性に応じた条件付き早期承認などを可能にする「医薬品，医療機器等の品質，有効性及び安全性の確保等に関する法律（医薬品医療機器等法，いわゆる改正薬事法)」が成立，再生医療はこれに特化した法的位置づけで扱われることになった。これらの法律に関連する政省令等の整備が 11 月までに行われ，法律が正式に施行される。

最終目標を再生医療の産業化とした場合，研究開発の振興と合理的な規制の整備と運用は効率，効果的に目標達成に向けて推進・加速を図るための車の両輪である。この両輪共に国際的優位性を確保していくことが肝要である。

図 細胞・組織利用医薬品等の開発から使用までの各種のあり方



法規制

ほうきせい

legislative regulation

関連用語　薬事規制当局（●）　医事規制当局（●）　公定書・基準（●）　指針（●）　審議会（●）

日本において，ヒトまたは動物の細胞に培養その他の加工を施したものを用いた再生医療等（再生医療または細胞治療）を規制する根拠となる法律には，「医療」に関するものと「製品」に関するものの二つがある。医師・歯科医師が自らの患者に「医療」を施すことを目的に，ヒトまたは動物の細胞に医師・歯科医師が自ら加工を施し，これを患者に投与することは，『医師法』『医療法』等の医事関連法規の下で行われている。『再生医療等の安全性の確保等に関する法律』施行後は，医師・歯科医師は細胞の加工を外部の「特定細胞加工物製造業者」に委託することが可能となる一方で，その区分に応じて，再生医療等提供計画を厚生労働大臣等に提出しなければならない。

一方，ヒトまたは動物の細胞に培養その他の加工を施し，再生医療等に用いられることを目的とした製品（再生医療等製品~~〔細胞・組織加工医薬品等〕~~）を製造販売する場合には，『薬事法』の規制を受け，薬事法に基づき，品質，有効性および安全性を審査のうえ，厚生労働省の製造販売承認を受けなければならない。2013年改正の『薬事法』の施行にともない，法律名が『医薬品，医療機器等の品質，有効性及び安全性の確保等に関する法律』（略称『医薬品医療機器等法』）に変更されるとともに，再生医療等製品（遺伝子治療用製品を含む）は，医薬品からも医療機器からも独立した第3のカテゴリーとして分類される。再生医療等製品のうち，申請に係る再生医療等製品が均一でない場合，治験により効能，効果または性能を有すると推定され，安全性の確認が行われたものは条件および期限付製造販売承認を得ることができるようになるなど，特別な規制が適用される。

なお，欧米では緊急時や治験等の例外を除き，ヒトまたは動物の細胞に培養その他の加工を施したものを臨床適用する場合には，「医療」か「製品」かの区別なく，その有効性，安全性および品質について品目ごとに規制当局の審査（欧州では通常品目は欧州医薬品庁〔EMA〕，病院内等で個々の患者に限定的に使用されるものは各国規制当局）を受け承認を得なければならない。

［佐藤 大作，佐藤 陽治］

薬事規制当局

やくじきせいとうきょく

drug regulatory authority

関連用語　法規制（●）　医事規制当局（●）　公定書・基準（●）　指針（●）　審議会（●）

薬事法に基づく規制を担当する組織。薬事法を所管する厚生労働省医薬食品局は薬事規制当局である。同様に，薬事法上の委任を受けて厚生労働大臣の権限の一部である医薬品等（再生医療等製品を含む）の製造販売承認審査，安全対策，GMP調査等の業務を行う独立行政法人医薬品医療機器総合機構（PMDA）も，薬事規制当局ということができる。日本では，製造販売業の許可や監督等の業務は薬事法上，都道府県知事等の地方自治体が担っており，都道府県庁等も同様に薬事規制当局である。

なお，米国では食品医薬品局（FDA），EUでは欧州医薬品庁（EMA）が再生医療等製品に関する中心的な薬事規制当局である。また，EU加盟各国政府には個々に薬事規制当局が存在し，再生医療等製品については製造（GMP等）および臨床試験に関する審査・助言等を行っている。

［佐藤 大作，佐藤 陽治］

医事規制当局

いじきせいとうきょく

medical regulatory authority

関連用語　法規制（●）　薬事規制当局（●）　公定書・基準（●）　指針（●）　審議会（●）

日本での医療行為としての再生医療等（再生医療・細胞治療）に必要な規制は厚生労働省が行っている。再生医療を含む医行為に関する規制を行う『医師法』，『医療法』，『再生医療等の安全性確保等に関する法律』等を所管し，規制権限を有するのは厚生労働省医政局および同省健康局であり，医事規制当局という。再生医療等の臨床研究（がんを対象にした細胞治療以外）は厚生労働省医政局が所管する一方，がんを対象にした細胞治療は健康局が所管している。なお，臓器移植および造血幹細胞移植は健康局の所管である。『再生医療等の安全性の確保等に関する法律』に基づく再生医療等提供計画の提出，再生医療等委員会の認定等は，同省医政局および厚生労働省地方厚生局が所管することとなる。

日本では，病院等の開設許可や監督等の業務は医療法上も都道府県知事等の地方自治体が担っており，都道府県庁等も同様に医事規制当局である。

［佐藤 大作，佐藤 陽治］