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Murine model for evaluating iPS-technology feasibility in age-related functional declines or diseases

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Introduction

The generation of pluripotent stem cells (iPSCs) open the possibility to personalized cell therapy. When applying the iPSCs for regenerative therapy in elderly patients, it is necessary to establish the iPSCs from elderly patients themselves then differentiate the iPSCs to specific cell types for transplantation treatments. The current study is to establish the murine model of aged-iPSCs then to evaluate the feasibility of applying aged-iPSCs for the treatment of incurable diseases of elderly patients.

The generation of induced pluripotent stem cells (iPSCs) from murine and human somatic cells through forced expression of defined four transcription factors (Oct3/4, Sox2, Klf4 and c-Myc) [1-4] constitutes a major breakthrough in regenerative biology. This technology opens the exciting possibilities in medical research and medical practice [5-8]. Recently, complex disorders such as schizophrenia with high heritability was analyzed by using patient-derived iPSCs [9].

The iPS-technology also opens the possibility of personalized cell therapies for treating human disease and/or repairing the damaged tissues of elderly patients. To treat damaged tissues or repair organs in elderly patients, it will be necessary to establish iPSCs from the tissues of patients themselves.

To determine the feasibility of using this technology with elderly patients, the unsolved question here is that if it was indeed possible to establish iPSCs from the tissues of aged somatic cells and the pluripotency of the aged-iPSCs. Hence, our recent works were about the establishment of iPSCs from aged mice (aged-iPSCs) and the possibility of applying the aged-iPSCs for regenerative medicine [10].

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1) Establishment of iPSCs from aged mouse

Since the BM-derived myeloid (BM-M) cells cultured in the presence of granulocyte macrophage-colony stimulating factor (GM-CSF) can be actively proliferating, BM-M cells of aged C57BL/6 mice were used for iPSCs establishing. The efficiency of iPSC generated from aged mice was investigated. MEF cells, BM-M cells from 2-month-old C57BL/6 mice were used as control and compared with 23-month-old BM-M C57BL/6. In both MEF cells and BM-M cells of 2-month-old mice, iPS colonies emerged approximately 15 days after transduction, although, the number of colonies from MEF cells increased more rapidly than those from 2-month-old BM-M cells. As for 23-month-old BM-M cells, colonies appeared 30 days after transduction. The colony number was less than those obtained from MEF cells or 2-month-old BM-M cells. These results indicated that efficiency to establish aged-iPSCs is lower than that of young mice（Fig.1）.

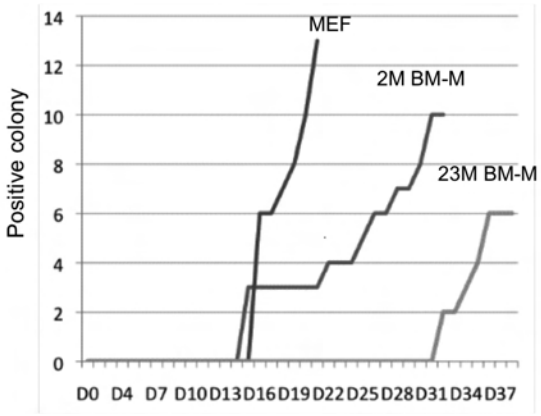

Fig. 1

In order to establish murine model of stem cell therapy for elderly patients, the C57BL/6 mice carrying GFP marker were selected. GFP will be useful marker to discriminates transplanted iPSCs from receipient syngeneic mice. The aged-iPSCs were established by using BM cells of 21-month-old EGFP-C57BL/6 mice. Cells were cultured for 4 days in the presence of GM-CSF then treated

twice with four reprogramming factors. Two clones (1 and 2) were picked up and expanded. Aged-iPSCs were compared with iPSCs from MEF (MEF-iPSCs) (Fig.2).

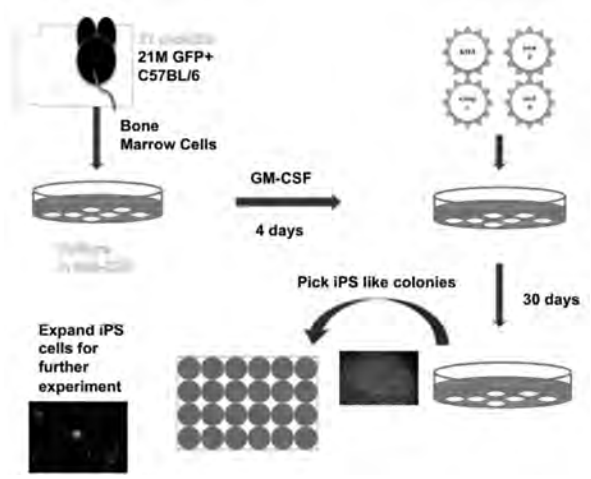

Fig. 2

2) Characterization of aged-iPSC and MEF-iPSCs?

If aged-iPSCs were rejuvenated as same as MEF-iPSCs, the gene expression of aged- iPSCs may be similar to MEF-iPSCs. We analyzed pluripotent genes expression by RT-PCR and epigenetic status by Chip assay. Our results showed that both aged-iPSCs and MEF-iPSC strongly express SSEA-1 and Pou5f1, and also showed the strong alkaline phosphatase (AP) activity. The gene expressions of Nanog and Pou5f1 in aged-iPSC were as same as MEF-iPSC (Table 1).

	BMD4	Aged-iPS	MEF-iPS
Tnf	++	−+	+
IL-1β	++	−	−
CCL-7	+	−	−
C/EBPα	++	−	−
Pu-1	++	−	−
Nanog	−	++	++
Pou5f	−	+	+
Fgf-4	−	+	+
Esg1	−	+	+
Cripto	−	+++	+++

Table. 1

3) Do aged-iPSCs differentiate as MEF-iPSCs?

Aged-iPSCs (1×107) were transplanted to the dorsal flank of syngeneic C57BL/6 mice. After 21 days, distinct teratoma was observed and collected then embedded in OCT compound. Various tissues belonging to three germ layers (endoderm, mesoderm and ectoderm) can be found in the teratoma. They were positively stained with alpha-smooth muscle actin (mesoderm), α-fetoprotein (endoderm) and neurofilament H (ectoderm), respectively. These evidences indicate that aged-iPSCs can be differentiated into cells belonging to different germ layers and make tissues in vivo (Fig.3).

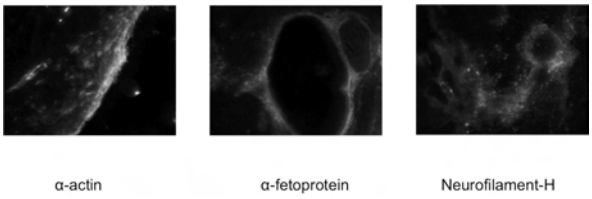

Fig. 3

Then the aged-iPSCs were analyzed by *in vitro* differentiation to three germ layers. Aged-iPSCs were hanging drop-cultured for 8 days to induce the embryoid body (EB). The EB were transferred to 24 wells for the spontaneous differentiation. After differentiation, the cells were stained with tissue-specific antibodies, and cells belonging three germ layers can be detected (Fig. 4). Currently we are trying to differentiate aged-iPSCs in vitro to various tissues.

Fig. 4

In order to investigate whether aged-iPSCs grow normally during development, chimera technique was applied. Aged-iPSCs were aggregated with embryos of ICR to make aged-iPSCs/ICR chimera mice (Fig.5). Although we could get several chimera mice, some chimera mice were dead in the uterus of pregnant mice before delivery. We are currently investigating the causes of deaths.

Our current trial to produce germ line transmission from aged-iPSCs (21 M old C57BL/6 mice) and examine the life span of these mice will answer the question of aging itself and/or usefulness of iPSCs for future regenerative medicine.

Fig. 5 Chimera 1month

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