Selection of Pure Differentiated cells for Safe Regenerative Medicine

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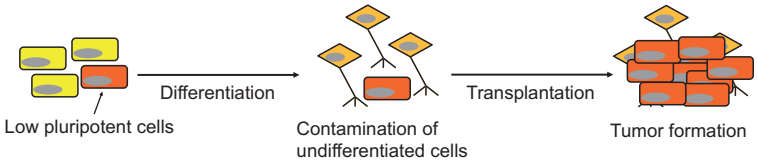
Introduction

Problems of differentiated cells for regenerative medicine and drug discovery

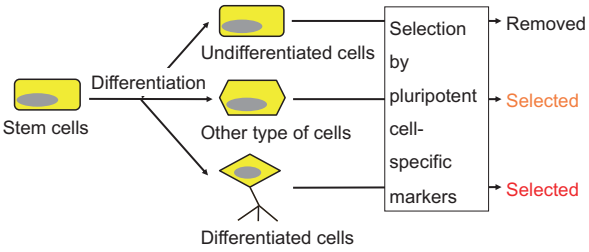
- Contamination of cells with low pluripotency during iPSC production and pluripotent stem cell maintenance
- Inefficient differentiation of the low pluripotent cells
- Undifferentiated cells have a risk of tumor formation and unexpected function



Problems of conventional methods

Cell isolation using pluripotent cell-specific markers

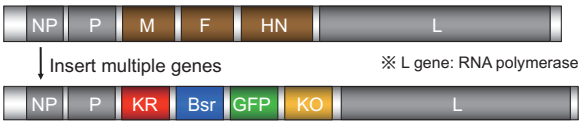
- Can't remove cells differentiated into other type of cells
- Needs high cost in cell sorting



Our technologies to solve the problems

Persistent Sendai virus vector (SeVdp vector)

Nishimura K. *et al.* (2011) *J. Biol. Chem.*

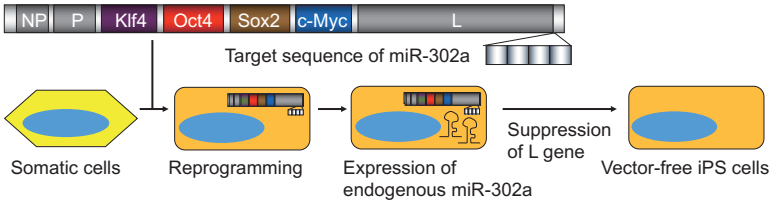


- Persistent expression of multiple genes
- No integration of vector genome into host chromosome
- Artificial removal of vector by suppression of L gene

Auto-erasable SeVdp vector

Nishimura K. *et al.* (2017) *Stem Cell Res.*

※ miR-302a : Pluripotent stem cell-specific miRNA



Automatic removal of a vector using cell type-specific miRNA

Purpose

Using our persistent and auto-erasable vector, establish a system for selection of pure differentiated cells

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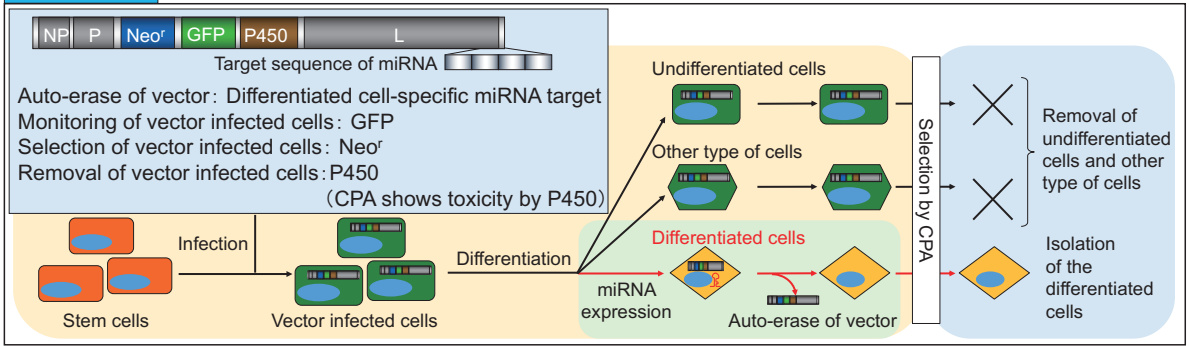
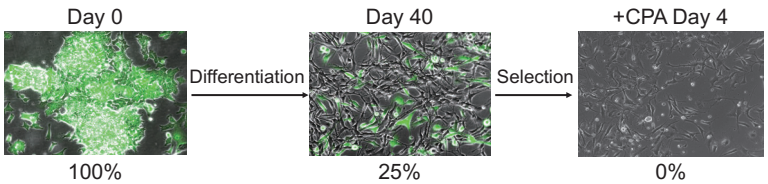
Regenerative medicine and drug discovery

Methods



Results

Use neural stem cell (NSC) differentiation from ES cell as a model

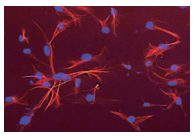


Linage differentiation

Differentiate into neural linage cells (Neuron, Astrocyte etc.)

Turmorigenicity

No tumor formation after transplantation into mouse brain



Nestin: NSC marker

We succeeded to select pure NSCs by auto-erasable SeVdp vector with NSC-specific miRNA target sequence

Perspectives

Additional expression of differentiation-inducing gene(s) will achieve both promotion of differentiation and selection of differentiated cells

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Enable us to acquire pure differentiated cells even if difficult to induce

Characteristics of this technology

- Differentiate after introduction of the vector to all of the cells
  →Select only differentiated cells
- Simple selection by drug addition
  →Large-scale selection with low cost
- Complete removal of vector genome from the cells
  →Usable for many application

实现安全再生医疗的高纯度分化细胞选择法

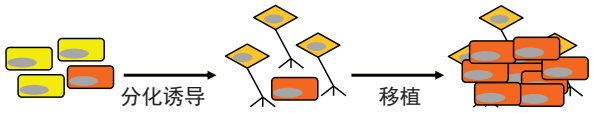
筑波大学　医学医疗系　基因控制学　　西村　健

University of Tsukuba

背景

旨在将使用iPS细胞等诱导的分化细胞应用于再生医疗和药物研发的课题

- 在iPS细胞诱导及培养维持时会混入多能性差的干细胞
- 多能性差的干细胞分化能力弱
- 残留未分化细胞等的混入会有导致肿瘤形成的风险和对药物试验的影响

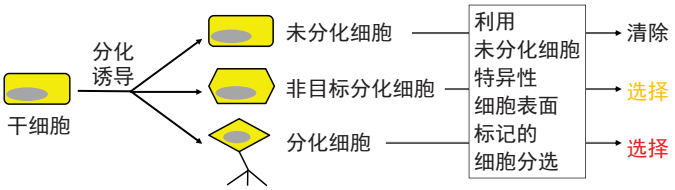


多能性差的干细胞的混入　　分化诱导的组织中混入未分化细胞等　　移植后形成肿瘤

传统方法的问题

开发利用未分化细胞特异性标记的细胞分选方法等

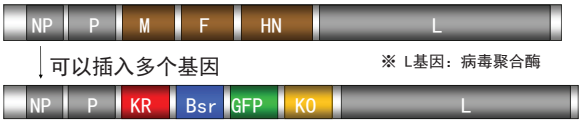
问题　无法清除分化为非目标细胞的细胞
难以扩大分选的规模



解决问题的技术

持续表达型仙台病毒载体（SeVdp载体）技术

Nishimura K. *et al.* (2011) *J. Biol. Chem.*

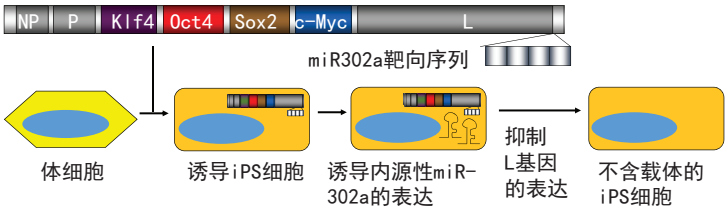


- 只要令其感染一次，就能持续表达多个基因
- 因为不会将载体基因组插入染色体中，所以安全性高
- 通过抑制L基因的表达，可以人工清除持续感染的载体

自动清除型SeVdp载体技术

Nishimura K. *et al.* (2017) *Stem Cell Res.*

※ miR-302a：未分化细胞特异性miRNA



利用细胞特异性miRNA，可以自动清除载体

目的

有效利用我们的可以在持续感染的同时利用细胞特异性自动清除的自动清除型SeVdp载体系统，确立轻松分选高纯度分化细胞的方法

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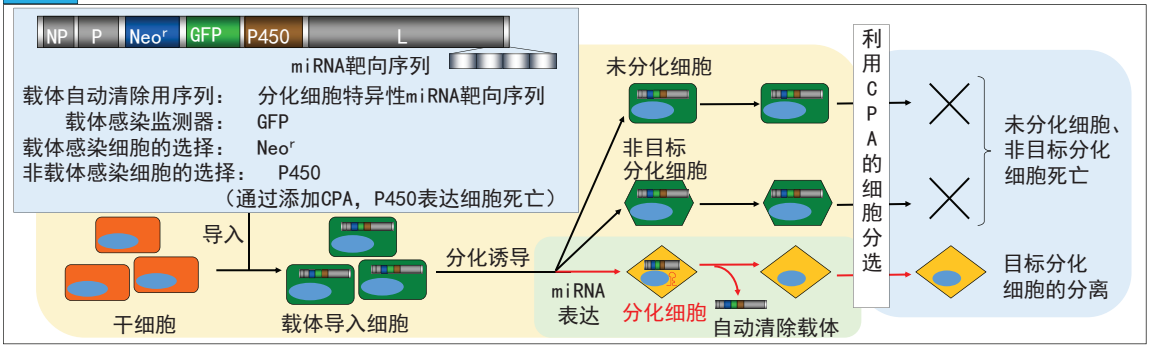
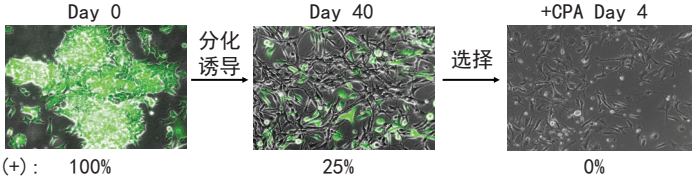
向再生医疗和药物研发提供安全的分化细胞

方法



结果

将从iPS细胞诱导神经干细胞的系统作为模型，构建分化细胞选择系统

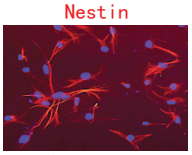


分化能力

分化为神经系统细胞（神经细胞、星形胶质细胞等）

致肿瘤性

即使移植到小鼠脑中等，也不会形成肿瘤



Nestin：神经干细胞标记蛋白

通过利用神经干细胞特异性miRNA的自动清除型SeVdp载体，成功实现了神经干细胞的选择

今后的应用

通过另外插入分化诱导基因，只要让一个载体感染一次，就能提高分化诱导效率并实现细胞分选

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现已可以对难以诱导分化的细胞进行分化诱导和选择了

本技术的特征

- 将选择用载体导入所有细胞后进行分化诱导
  →只对分化细胞进行准确的选择
- 只添加药剂，就能选择细胞
  →可以廉价地扩大规模
- 从被选择的细胞中自动清除载体
  →可以直接用于各种各样的应用