Application Note for PG-007, PG-008, and PG-010

PeptiGrowth

Differentiation induction of iPS-NKT cells

Summary

✓ With PG-peptides,PG-007,008, and PG-010, the first differentiation stage for HSPCs from iPSCs was shortened by one day, and more than twice the number of HSPCs were harvested compared to the control using VEGF, CHIR99021, and TPO.

✓ PG-peptides were effective at lower concentrations than VEGF(1/10), CHIR99021(1/2500), and TPO(1/5).

✓ The differentiation efficiency into NKT cells and the functionality of the obtained NKT cells with PG-peptides were comparable to the control, with a significant increase in the total number of harvested cells.

Outline

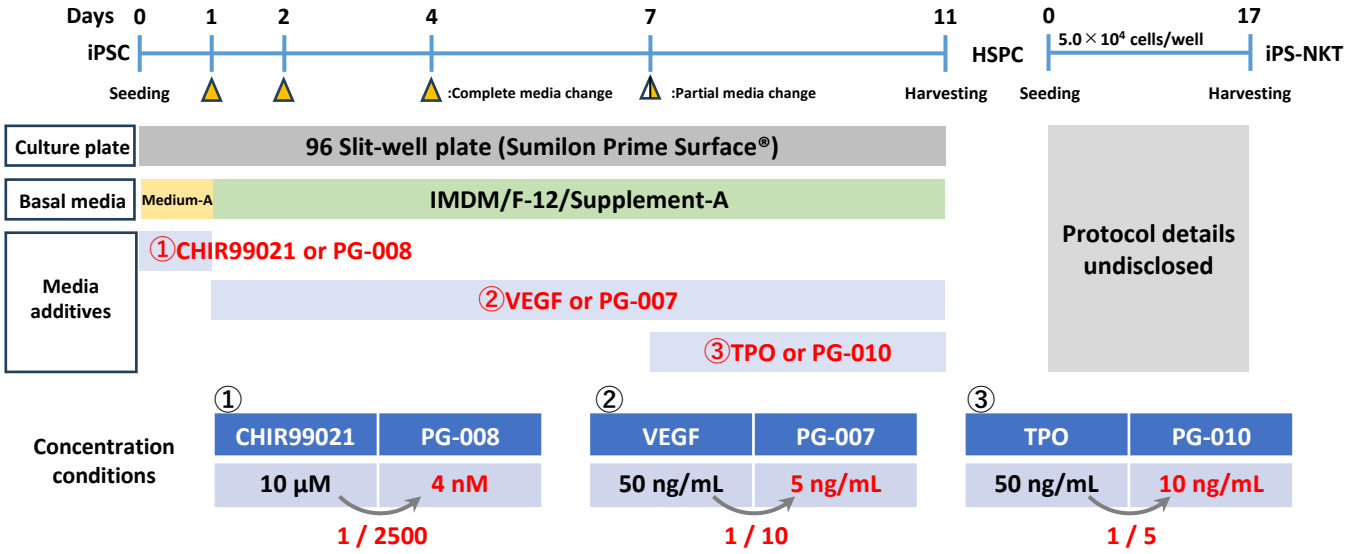
PeptiGrowth Inc. has been developing synthetic peptide growth factors (PG-peptides) with activity equivalent to growth factors and cytokines. Among them, PG-peptides for vascular endothelial growth factor (VEGF) (**product code: PG-007**), Wnt3a (**product code: PG-008**), and thrombopoietin (TPO) (**product code: PG-010**) can be utilized to produce natural killer T (NKT) cells through the differentiation from induced pluripotent stem cells (iPSCs) to hematopoietic stem and progenitor cells (HSPCs).

Herein, we demonstrated the differentiation of iPSCs into NKT cells through differentiation into HSPCs using PG-007, PG-008, and PG-010, and compared the productivity of HSPCs and NKT cells and the functionality of NKT cells with the control condition using VEGF, CHIR99021 (Wnt3a agonist), and TPO.

Protocol Overview

1st differentiation stage
(iPSCs→Hematopoietic stem progenitor cells: HSPCs)

2nd differentiation stage
(HSPCs→iPS-NKT cells)

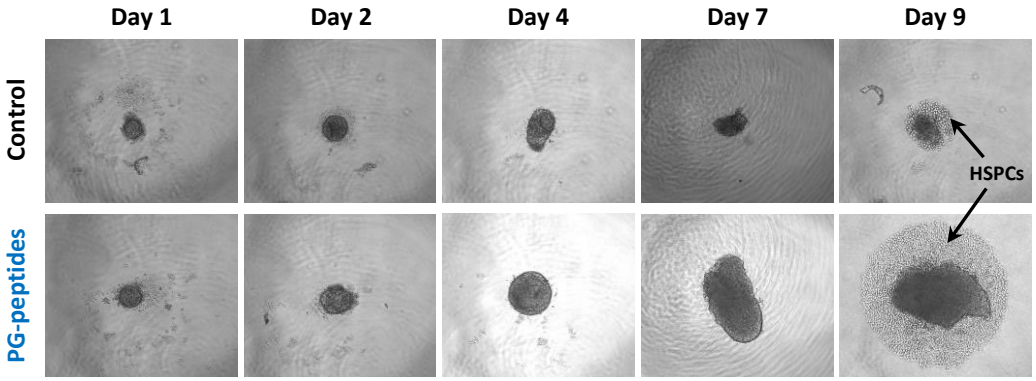


The 2nd differentiation stage was conducted under identical conditions for both the control condition and the condition using PG-peptides.

The experiment was carried out at Center for Integrative Medical Sciences Laboratory for Developmental Genetics in RIKEN.

Results of the 1st differentiation stage *(iPSCs→HSPCs)*

Microscopic observation on differentiation process of iPSCs into HSPCs



✓ With PG-peptides, the growth of cell spheroids as well as the differentiation of iPSCs into HSPCs proceeded faster than under the control condition. As a result, the 1st differentiation period was **shortened by one day, totaling 10 days**

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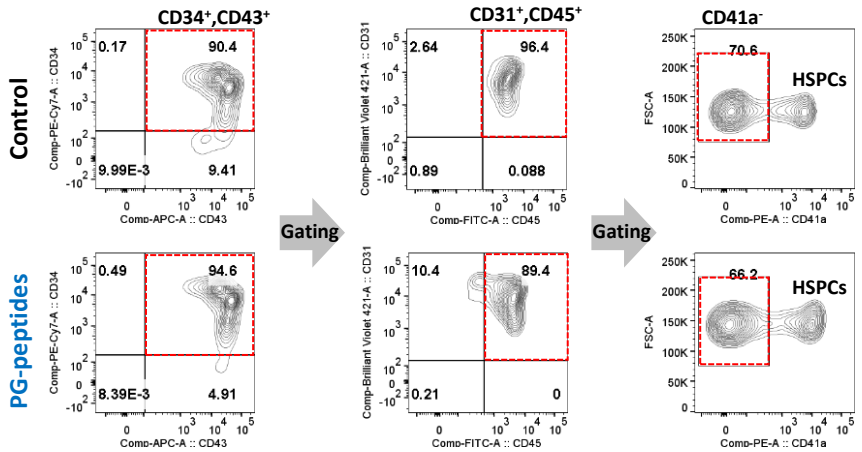
Results of the 1st differentiation stage (iPSCs→HSPCs) -continued from previous page-

HSPC surface marker measurements and cell counting



Number of harvested cells

0.65 × 10⁶ cells

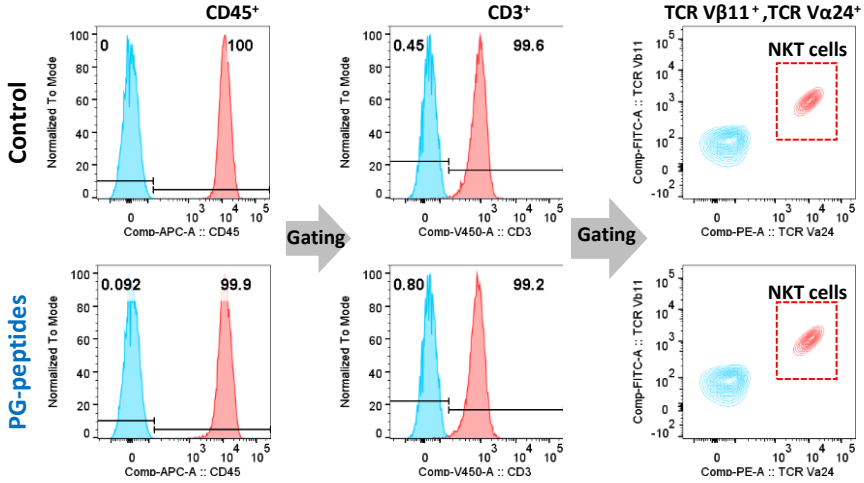
Increased!

1.6 × 10⁶ cells

✓ The differentiation efficiency of iPSCs into HSPCs using PG-peptides was comparable to the control condition. With PG-peptides, the number of harvested HSPCs significantly increased, and **more than twice the number of HSPCs were harvested** on Day 10 compared to the control condition on Day 11.

Results of the 2nd differentiation stage (HSPCs→NKT cells)

NKT cell surface marker measurements and cell counting



Number of harvested cells

3.4 × 10⁶ cells

Proliferation ratio*

67x

Increased!

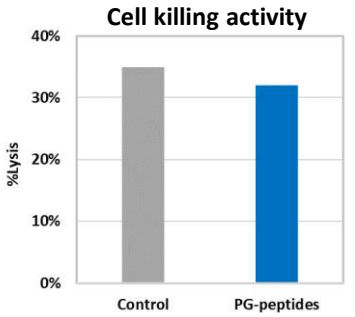
7.5 × 10⁶ cells

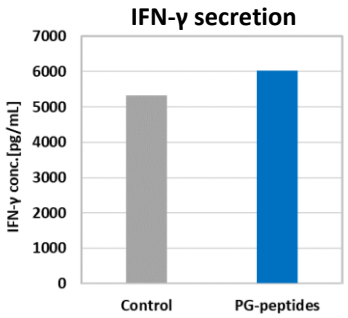
150 x

*from the start of the 2nd differentiation stage (5.0 × 10⁴ cells)

✓ With PG-peptides, the differentiation efficiency of NKT cells from HSPCs was comparable to the control condition, and **the number of harvested NKT cells significantly increased.**

Functionality assessment of NKT cells





✓ Both NKT cells generated under the control condition and those generated using PG-peptides showed **comparable cell killing activity against cancer cells in vitro and levels of IFN-γ secretion.**

✓ In summary, with PG-007, 008, and PG-010, we were able to **increase the efficiency of differentiation of iPS-NKT cells** with this protocol, **shortening the process by 1 day**.

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